Eesti Maaülikool
Estonian University of Life Sciences

ON THE RELATIONSHIPS BETWEEN PLANT LITTER AND THE CARBON AND NITROGEN CYCLES IN EUROPEAN FOREST ECOSYSTEMS

EUROOPA METSAÖKOSÜSTEEMIDE SÜSINIKU- JA LÄMMASTIKURINGE SEOSED TAIMSE VARISEGA

MIGUEL PORTILLO ESTRADA

A Thesis
for applying for the degree of Doctor of Philosophy
in Ecophysiology

Väitekiri
filosoofiadoktori kraadi taotlemiseks ökofüsioloogia erialal

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To my beloved Clotilde

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following research papers:

I Morillas L, Gallardo A, Portillo-Estrada M, Covelo F (2012). Nutritional status of *Quercus suber* populations under contrasting tree dieback. Forestry, 85: 369–378.

II Portillo-Estrada M, Korhonen JFJ, Pihlatie M, Pumpanen J, Frumau AKF, Morillas L, Tosens T, Niinemets Ü (2013) Inter- and intra-annual variations in canopy fine litterfall and carbon and nitrogen inputs to the forest floor in two European coniferous forests. Annals of Forest Science doi: 10.1007/s13595-013-0273-0 (in press)

III Portillo-Estrada M, Noe SM, Noe B (2013) Litterfall dynamics and VOC emissions from soil litter layer. Geophysical Research Abstracts 15:EGU2013-10616.

IV Noe SM, Kimmel V, Hüve K, Copolovici L, Portillo-Estrada M, Püttsepp Ü, Jõgiste K, Niinemets Ü, Hörtnagl L, Wohlfahrt G (2011) Ecosystem-scale biosphere–atmosphere interactions of a hemiboreal mixed forest stand at Järvselja, Estonia. Forest Ecology and Management 262:71–81.

V Carter MS, Larsen KS, Emmett B, Estiarte M, Field C, Leith ID, Lund M, Meijide A, Mills RTE, Niinemets Ü, Peñuelas J, Portillo-Estrada M, Schmidt IK, Selsted MB, Sheppard LJ, Sowerby A, Tietema A, Beier C (2012) Synthesizing greenhouse gas fluxes across nine European peatlands and shrublands – responses to climatic and environmental changes. Biogeosciences 9:3739–3755.

The contribution of the authors to the papers:

Paper	Idea and study design	Data collection	Data analysis	Manuscript preparation
I	AG	All	All	All
II	MPE, ÜN, TT	JK, MP, JP, AF	MPE, JK, LM	All
III	All	All	All	All
IV	SN	All	All	All
V	MC	All	All	All

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ABBREVIATIONS

VOC	Volatile organic compounds
GHG	Greenhouse gas
NR	Nitrogen resorption
NRE	Nitrogen resorption efficiency
NRP	Nitrogen resorption proficiency
NRR	Nitrogen resorption rate

1. INTRODUCTION

1.1. Importance of carbon and nitrogen in ecosystems

There are just six elements: carbon (C), nitrogen (N), oxygen (O), hydrogen (H), phosphorus (P) and sulfur (S); that are the building blocks of all living organisms on Earth. Carbon and nitrogen are the major elements that are cycled in ecosystems. They are important macronutrients in the biosphere, and fulfill very different functions in ecosystems and belong to the major elements studied in biogeochemistry; together with hydrogen, oxygen, phosphorus and sulfur. These six elements build the bulk of both plant and animal tissues.

Carbon is essential for life because it makes up the skeleton of macromolecules that create the storage matrix for N and other nutrients (Berg and McClaugherty 2008) and gives structure to all living organisms. Plants fix atmospheric carbon in form of carbon dioxide (CO_2) by photosynthesis. It is the most important process made by plants on Earth and it is the main flux of C from atmosphere to plants.

All organisms require N to build amino acids which act as building blocks for proteins, nucleic acids, and in the case of bacteria also cell wall. Molecular nitrogen (N_2) is the most abundant molecule in the atmosphere, but it is not directly usable by plants. Plants take up nutrients in form of ions. Ammonium (NH_4^+) and nitrate (NO_3^-) are the major source of N taken up by the root system. The N_2 reduction to ammonia (NH_3) by nitrogen-fixing bacteria is the major process to make N available for biosphere (Postgate 1998). From this transformation, the N starts its cycle in ecosystems from the soil pool, and becoming assimilable by bacteria and plants. Despite N_2 is abundant in atmosphere, N_2 fixation is bottle neck for the N availability in ecosystems. Therefore N is a limiting factor for plant growth. Nitrogen availability is usually related to the speed of key processes and nutrient fluxes between the ecosystem pools, therefore the importance of studying its biogeochemical cycle.

1.2. The carbon and nitrogen cycle in forest ecosystems

Studies in biogeochemistry focus on the nutrient cycling between the organic and inorganic states of the elements, and the speed of the transforming processes. Living organisms maintain a C to N ratio in their biomass to ensure correctly functioning of their metabolism (for example: the nitrogen content of amino acids is 15-17 %) (Bäck *et al.* 2013). Therefore C and N cycles are tightly linked. Since plant biomass contain a certain amount of C and N, these elements are transferred to subsequent pools in the forest nutrient cycle.

The soil-biosphere-atmosphere relationships with emphasis in the plant litter role is described in Figure 1. The inlet to the soil-plant N cycle in forest would correspond to the atmospheric nitrogen (N_2) fixation by bacteria together with atmospheric wet and dry deposition of nitrogen oxides (NO_X), ammonia (NH_3), and nitric acid (HNO_3). On the other hand, plants assimilate atmospheric C in the form of carbon dioxide (CO_2) through photosynthesis, and emit a part of it because of cell respiration. Plants emit a substantial fraction of their assimilated carbon (possibly up to 10 % (Peñuelas and Llusià 2004)) in the form of volatile organic compounds (VOCs).

The return of nutrients from the plants to the soil is driven by litterfall. The nutrient content in litterfall is the result of the nutrients in senescent plant material minus the nutrient resorption, also called retranslocation. The nutrient resorption is a process taking place inside the plant individual, which permits the preservation of a portion of the senescent nutrients. A portion of the nutrients in senescent matter are broken down, transported and assimilated in other longer living storage cells. Nutrient storage is performed by building proteins (Wetzel *et al.* 1989), and it occurs in: storage cells under the inner bark of twigs, outer sapwood of the main stem, and in corresponding root tissues (Millard and Proe 1991). In this way, nutrients can be re-used to create or maintain living tissues. The resorption of N and other elements like P, K, Ca, Mg, Mn are important to the plant because they usually are scarce in the ecosystem. The remaining biomass from the senescence process will be shed from the plant and becomes litter. In addition to the ideal process, nutrient resorption is not completely achieved in all cases before the litterfall. Premature plant material abscission occurs usually because of wind

damage, snow load on branches, early frosts, herbivory, etc (Portillo-Estrada *et al.* 2013). Therefore usually, litterfall is not purely composed of litter in its optimal stage of resorption. The resultant litterfall (from above-ground or below-ground) is then accumulated in the soil and starts its process of decomposition.

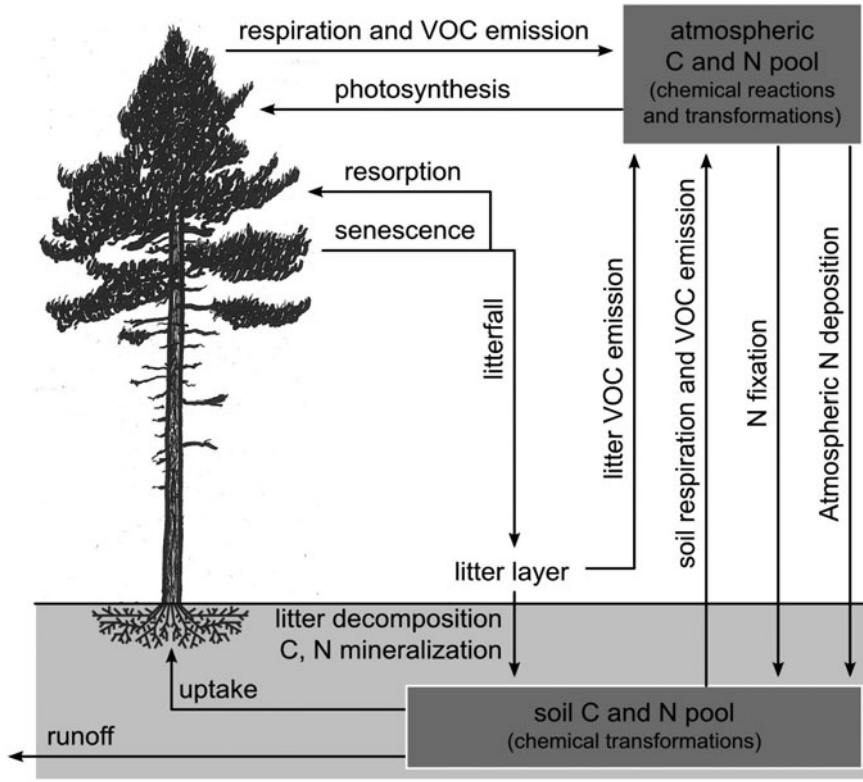

Figure 1. Scheme of the carbon and nitrogen cycles in forests. Litter and related processes include both above- and below-ground litter.

Dead matter is broken down into simpler structures and molecules. Then, they undergo chemical transformations until the mineralization of nutrients. During the process, the VOC stored in the litter tissues and resins are emitted to the atmosphere driven by temperature and the action of decomposers. The litter decomposition is driven by the litter quality (chemistry, toughness, specific leaf area, etc.), the climatic conditions (temperature, moisture, etc.), and the diversity and abundance of the decomposer community (bacteria, fungi, macroinvertebrates, etc.). During litter decomposition processes, carbon and nitrogen pass

several stages of organic substances until the final mineralization. Litter decomposition also releases important quantities of CO_2, methane (CH_4), and nitrogen-based gases (Berg and McClaugherty 2008). Once the carbon and nitrogen are mineralized, soil nutrient pool further undergo chemical transformations, which also release CO_2, CH_4, nitrous oxide (N_2O), N_2, etc. These depend on soil fauna, moisture, soil texture, and other soil and climatic characteristics. The soil nutrient pool serves as main source for plants and is accessible to them via the root system uptake.

Nutrient loss from the soil pool and thus the forest nutrient cycling, occur when dissolved nutrients (organic forms, NO_3^-, NH_4^+) escape from the system through superficial runoff and leaching to deeper soil layers, far from the rhizosphere.

1.3. Litterfall dynamics in different European forests

Deciduous trees in European temperate forests are, for example: English oak (*Quercus robur* L.), European beech (*Fagus sylvatica* L.), Norway maple (*Acer platanoides* L.), and silver birch (*Betula pendula* Roth). They build one-season leaves, which grow and senesce within some months. In autumn, cold temperatures and decreasing light are unfavorable conditions for net primary production. Then, leaves senesce and fall to the topsoil, generating the annual maximum N input to these soils (excluding sites with high atmospheric N deposition).

In boreal forests, where evergreen conifers such as Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) Karst.) and firs (*Abies sp.* Mill.) predominate, the dynamic is different. They support several cohorts of leaves on their canopy lasting several years. Foliage longevity in these species is variable within the canopy: new leaves are produced in spring and old leaves are abscissed during the whole year, although there are leaf litter production peaks (Portillo-Estrada *et al.* 2013).

Hemiboreal mixed forests support deciduous and evergreen species. The input of nutrients to the soil from litterfall is produced as a combination of the litterfall dynamics of both types of species.

The evergreen broad-leaved Mediterranean species such as cork oak (*Quercus suber* L.), olive (*Olea europaea* L.), mastic (*Pistacia lentiscus* L.), and oleander (*Nerium oleander* L.) behave as deciduous trees regarding litterfall. They conserve leaves for more than one growing season, keeping a green canopy. The shedding of leaves occurs during the hot and dry summer months.

2. REVIEW OF THE LITERATURE

2.1. Studying the forest N and C cycling

Biogeochemical cycles enable the transport of elements from one to another compartment of the ecosystem through matter fluxes. By accompanying processes the elements are transformed between inorganic and organic states. The transport and transformation of matter enable the utilization of nutrients by organisms, satisfying their specific needs.

The study of the biogeochemical cycle of a nutrient can be as complex as scientists' capacities, time, man-power and funding resources permit it. Usually, the best strategy is to focus in a part of the cycle to acquire more knowledge about a specific subject. Even though, many work is focused on the N cycling in forest ecosystems generally, see examples in Bormann *et al.* (1977), Tsutsumi (1987), Korhonen *et al.* (2013), the focus of this work has been the role of plant litter in the forest nutrient cycle.

Plant litter production and decomposition is one of the key processes unifying soil-biosphere-atmosphere interactions. There are many studies on plant litterfall for assessing the cycling of matter and specific elements in terrestrial ecosystems. Litter decomposition, in special leaf litter has been a recurrent topic as scientists gained more knowledge about the litter quality, soil chemistry processes and soil fauna. Current outstanding scientists in these topics are John D. Aber, Rien Aerts, J. Hans C. Cornelissen, Björn Berg, Grégoire Freschet, Charles McClaugherty, Jerry M. Melillo, Cindy Prescott, and others.

2.2. Nitrogen deposition effects on forest nutrient cycling

The global N cycle can be altered in many ways by human activities. Magnani and co-workers promoted the idea that mankind controls the balance of temperate and boreal forests either directly by forest management or indirectly by N deposition (Magnani *et al.* 2007). Fossil fuel combustion, mineral fertilizers and livestock manures introduces new sources of N for the ecosystems. Among the N compounds, there are volatile forms of nitrogen which may be deposited onto the forest floor. These amounts of N directly lead to an excess load to the

ecosystems, producing an impact on the N cycling balance. Although natural atmospheric N deposition is crucial for keeping the N cycle balance, its increase can result on ecosystem eutrophication, acid rain, soil acidification, leaf damage, forest dieback, and loss of biodiversity (Gruber and Galloway 2008).

Anthropogenic N deposition into N-limited ecosystems is a major component of the so-called *global change*. During the twentieth century, anthropogenic N fixation doubled the global flux of N to the biosphere (Morillas *et al.* 2012). Moreover, N deposition rates are expected to increase another two or threefold before reaching a plateau (Galloway and Cowling 2002; Lamarque 2005; Vitousek *et al.* 1997).

Increased atmospheric N deposition to forest ecosystems was already identified in the 1980s as a major contributor to forest damage in many regions of Europe (Aber 1992; Nihlgard 1985; Schulze and Freer-Smith 1990). Fortunately, European policies reduced the emission of nitrogen-based gases lately. Estonia is among the top European countries in reduction of NH_3 and NO_X emissions since beginning of 1990s (Erisman *et al.* 2003), likely because of changes in the agricultural strategies and improvement of energy use efficiency.

2.3. Litterfall and volatile organic compounds emission

Biogenic volatile organic compounds are based on carbon skeletons. They perform two main functions for plants: they are information carriers in the chemical communication between individuals and they scavenge reactive chemicals like ozone. The release of VOCs is frequently associated to plant stress, for example: ecosystem-scale disturbances like flooding, hot waves, or insect outbreak.

As technology improved, it permitted to measure the volatile organic compounds (VOCs) emitted by plants, soil organic layer (litter layer) and soil organic matter. Volatile organic compounds have a low boiling point. This results into a high vapor pressure, which cause the molecules to evaporate or sublimate and diffuse into the atmosphere. Volatile organic compounds include a variety of ca. 30000 compounds, and include isoprene, terpene, alkanes, alkenes, alcohols, aldehydes, eters, esters and carboxylic acids (Jun-wen and Cao 2005; Peñuelas and Llusià

2004). The lifetime of hydrocarbons in atmosphere is short (Kesselmeier and Staudt 1999) (from seconds to hours); they easily react and undergo oxidation and photolysis (Hellén *et al.* 2004). Many scientists are making big efforts to understand the role and reasons of plant VOCs emission (Niinemets *et al.* 2004; Peñuelas and Llusià 2004; Rosenstiel *et al.* 2004). Scientists still discuss whether plant VOC emission is a metabolic *safety valve* against the accumulation of reactive oxygen species, or if it is determined by the intrinsic physicochemical characteristics of the compounds emitted. But generally, scientists agree that VOCs protect plants from extreme conditions, play a role in plant communication, and protects them from herbivores.

A large source of VOC emission is the forest soil. Volatile organic compounds from soil are emitted by living roots, above- and below-ground litter and microbes; where litter is the strongest source (Aaltonen 2012). The litter VOC emission is produced as decomposition takes place. Its emission rate has shown dependency to moisture and temperature (Aaltonen 2012).

2.4. Importance of studying greenhouse gas emissions

Among the volatile compounds emitted by forest soils, greenhouse gases (GHG) like CO_2, H_2O, CH_4 and N_2O are released to the atmosphere. The study of these emissions has a special interest to scientists because of the global warming situation where we live nowadays:

(1) Atmospheric concentration of carbon dioxide has being rising during the last 150 years. By February 2013, the reference measurements in Mauna Loa (Hawaii) estimated a tropospheric CO_2 concentration of 395.89 ppm (season-corrected trend value) (Tans and Keeling 2013), 10 ppm higher that when this thesis was started in summer 2008 (385.82 ppm CO_2). Carbon dioxide absorbs infrared and near-infrared radiation, and it slowly emits infrared radiation. In atmosphere, it is responsible for retaining the Earth's heat (Lacis *et al.* 2010). But the rapid increase in air CO_2 concentration is having a high impact in the heat balance of Earth.

(2) Water vapor is graded as the gas applying the strongest greenhouse effect (Lacis *et al.* 2010) due to its hydroxyl bond, which absorbs infrared radiation. The average lifetime of water vapor in atmosphere is around

10 days (Miao *et al.* 2001), and it functions as a heat transporter on Earth's surface.

(3) Methane is produced by microbes through anaerobic respiration. Methane has a greenhouse effect equivalent to 72 times that of CO_2 in a 20-year period (Forster *et al.* 2007). Oceans, boreal soils and permafrost areas are estimated to be large sources of CH_4. If global warming continues to happen, great amounts of CH_4 can be released to the atmosphere from these sources.

(4) During early 1970s, atmospheric scientists suggested that N_2O released to the atmosphere through denitrification of NO_3^- in soils and natural waters could cause the destruction of the ozone layer in the stratosphere (Bremner 1997). In recent decades, N_2O atmospheric concentration has been rising about 0.25 % per year (Wuebbles 2009). Nitrous oxide's average lifetime in the atmosphere is 120 years because its removal happens by photolysis and reaction with excited oxygen atoms in the middle and upper stratosphere (Wuebbles 2009). Its global warming potential is equivalent to 289 times that of CO_2 in a 20-year period (Forster *et al.* 2007).

2.5. Sensitivity of ecosystems to alterations in the nutrient cycling

A large number of the endangered plants (about 75 %) are confined to N poor soils, facing the risk of competitive exclusion by plants better adapted to use higher amounts of N (Nordin *et al.* 2005). Excess of N load to ecosystems leads to loss of biodiversity (Stevens *et al.* 2010).

Mediterranean ecosystems are usually recognized as hot-spots of biodiversity (Myers *et al.* 2000). However, the potential ecological effects of N deposition have been poorly investigated (Bobbink *et al.* 2010). The response of Mediterranean ecosystems to increased N availability depends on its ecological succession stage (Ochoa-Hueso *et al.* 2011). It may be faster and more severe in the early stages following a disturbance (fire, soil removal, etc.) when nitrophilous plants dominate (Aber *et al.* 2003; Vourlitis *et al.* 2007).

For temperate and boreal ecosystems in Europe, a general critical load of ~10 kg N ha^{-1} yr^{-1} (based on N leaching) has been proposed by Dise

and Wright (1995). In northern European boreal ecosystems, where the N deposition is low (~2 kg N ha^{-1} yr^{-1}), N addition experiments have shown that European temperate acidic grasslands are sensitive to increased N deposition, causing changes in species richness (Dupré *et al.* 2010; Stevens *et al.* 2004).

Peatlands are most abundant under the boreal and temperate climates. Finland and Sweden together account the 65 % of the European area of peatlands (Montanarella *et al.* 2009). Peatlands are areas poor in nutrients, where organic matter has been accumulated over the time. Therefore, they are a major storage of terrestrial C (Clymo *et al.* 1998; Gorham 1991). These ecosystems are sensitive to increase in soil temperature, because the carbon stored may be mobilized by bacteria and released to atmosphere. Land needs to be drained if peat soils are used for silviculture, cropland, grassland and peat extraction. Peatland drainage mobilizes soil nutrients, speeds up the turnover of C and N, changes the soil properties, enhances tree growth, and produces volatile compounds.

Another anthropogenic alteration to forests, like clearcutting, increased the amount of ammonium deposition reaching to the forest soil. Due to the lack of forest cover also the runoff of soil nutrients increased (Nieminen 1998).

Waterlogging naturally occurs in flat northern regions when, after long winters, accumulated snow melts down. Fluctuating water table creates constantly alternating aeration conditions and thus incomplete denitrification (Lamers *et al.* 2007), which has been identified as the major N_2O forming process in soil (Vor *et al.* 2003).

During the last decades, decision makers and policy increasingly demand national inventories of GHG emission, to develop appropriate strategies and mitigation options (Lamers *et al.* 2007). Climate change predictions suggest an increase of the air temperature in the northern regions of Europe for the coming years. Air and soil temperature is directly linked to soil bacterial activity. Temperature increase and amplitude and frequency of drought, flooding, heat wave, and storm events will lead to higher stress to plants (Reyer *et al.* 2013). These phenomena increase the soil emissions of CO_2, CH_4, N_2O, and stress-related VOCs.

3. AIMS OF THE STUDY

In this thesis, my major hypothesis is that the different pools and fluxes of nutrients in forest ecosystems are tightly connected. Therefore alterations to one pool or flux will be transmitted to subsequent pools and fluxes. Studying the amount of plant litter production and its content of C and N will provide information of both the processes forming it and the subsequent steps of the nutrient cycling. As an example: an increase in the N deposition onto a forest could be reflected in the N concentration of the foliage, and therefore reflected in the N resorption performance during senescence, and litterfall N concentration. Furthermore, as litterfall deeply effects soil nutrition, an alteration on litterfall C and N concentration could alter the speed of litter decomposition, N mineralization, and related volatile organic compounds emitted to the atmosphere.

This thesis studies the processes related to plant litter in the context of the C and N cycles. Processes which generate plant litter (such leaf senescence and nutrient resorption) to processes derived from the litterfall which imply the return of C and N to soil and atmosphere (litter volatile organic compound emission, nutrient mineralization, and soil nutrient chemical transformations and emission).

This thesis also evaluates the effect of increased N deposition and peatland drainage on the C and N cycles, with special attention to soil greenhouse gas emissions.

The key questions of the present thesis are:

1) To study the senescence and litterfall traits in contrasting climates and tree species in Europe. To characterize the amount and quality of the nutrient return to soil through litterfall (Paper I, II). The northern ecosystem, where there was a smaller annual average temperature, less N deposition and less fertile soils could generate a situation of more conservative strategy for the plants regarding nutrient resorption during leaf senescence. This would also lead to less amount of litterfall.

2) To determine the effects of atmospheric N deposition and contrasting climatic conditions on soil fertility, leaf N concentration, leaf senescence

and litter concentrations (Paper I, II). The speed of nutrient cycling could be slower in the more limited site on northern Europe, which presents less atmospheric N deposition, less soil N availability and less favorable growing conditions.

3) To assess sources and sinks of GHGs and VOCs in an Estonian hemiboreal forest (Paper III, IV).

4) To describe the effects of drainage on an Estonian peatland and its consequences to the C and N balance: soil N_2O, CO_2 and CH_4 emissions; and contribution to the global warming (Paper V). After a long-term action on the water table in a peatland, the properties of the soil and its chemical processes could change. This could modify the soil GHGs emission to a different status, more similar to a hemiboreal forest.

4. MATERIALS AND METHODS

4.1. Study sites

The studies were conducted in five sites along Europe (Table 1). The sites covered diverse representative climates and tree species present in Europe.

4.1.1. Hyytiälä (Finland)

The Hyytiälä Forestry Field Station (Kolari *et al.* 2009) is located in southern Finland. It supports a homogeneous monoculture Scots pine (*Pinus sylvestris* L.) stand. This species is dominant in more than half of the forest area in southern Finland. Stand management has been conducted according to the standard silvicultural guidelines for similar forest types in Finland. By now, the stand is half way through the rotation time. The site has a relatively flat topography.

4.1.2. Järvselja (Estonia)

The Järvselja Experimental Forest of Estonian University of Life Sciences is located in southeastern Estonia (Noe *et al.* 2011). Järvselja is situated in the hemiboreal forest zone with a moderately cool and moist climate. The length of the growing season (daily air temperature above 5 °C) averages between 170 and 180 days. In winter, 40–80 mm of the precipitation falls as snow. The site supports stands of deciduous and coniferous species, representative of the temperate to boreal transition. The stands presented different dominant tree species: (1) Norway spruce (*Picea Abies* (L.) Karst.), (2) Scots pine, and (3) Silver birch and Downy birch (*Betula pubescencs* Ehrh.). The oldest trees were 35 years old (Sims 2013) and had an average height of 18 m by 2009. The soil shows a thick raw humus layer (24 cm).

Table 1. Characteristics of study sites.

Site name	Coordinates	Climate	MAT	MAP	Species	Soil type	Soil pH
Hyytiälä	61° 51' N 24° 17' E 181 m.a.s.l.	Boreal	3.5	713	Scots pine	Haplic podzol	3.3
Järvselja	58° 16' 17'' N 27° 16' 13'' E 47 m.a.s.l.	Hemiboreal	5.0	600	Norway spruce Silver birch, Black alder Scots pine	Haplic gleysol (eutric)	5.5
Männikjärve peat bog	58° 52' 34'' N 26° 14' 09'' E 80 m.a.s.l.	Hemiboreal	4.6	889	Peat moss Scots pine	Histosol	3.4
Männikjärve pine bog forest	58° 52' 31'' N 26° 15' 35'' E 80 m.a.s.l.	Hemiboreal	6.0	889	Scots pine	Histic gleysol	2.2
Speulderbos	52° 15' N 05° 41' E 52 m.a.s.l.	Atlantic	9.6	889	Douglas fir	Orthic podzol	3.7
Los Alcornocales North Plot	36° 28' 43'' N 05° 38' 03'' W 349 m.a.s.l.	Mediterranean semi-continental	16	1200	Cork oak	Eutric cambisol	5.8
Los Alcornocales South Plot	36° 16' 27'' N 05° 34' 26'' W 117 m.a.s.l.	Mediterranean semi-continental	16	1000	Cork oak	Eutric cambisol	5.1

MAT is “mean annual temperature” (°C). MAP is “mean annual precipitation” (mm $year^{-1}$). Soil type after FAO classification.

As the site is located in the Lake Peipsi depression and bordered by wetland massifs, the soils are strongly groundwater influenced. The water table is ca. 45 cm during summer. Due to high clay content of the soil, the soil hydraulic conductivity is low, and because of the site's flat topography, soils become flooded in Spring when the snow cover melts down. Järvseja stands were drained about one century ago. The stand can be seen as a further step in the ecosystem succession happening during the drainage process taking place in Männikjärve site.

4.1.3. Männikjärve bog (Estonia)

Männikjärve bog (Carter *et al.* 2012) is in east-central Estonia. It is part of the Endla mire system and can be characterized as a mature raised bog with hummocks and hollows. In the hollows, pools of standing water are "perched"at levels above ground water and surface drainage in the surrounding terrain. It is colonized by peat moss (*Sphagnum sp*.), the pH of the water is acidic. It has an ombrotrophic water regime, and low nitrogen deposition, thus it is a site poor in nutrients with a slow nutrient cycling processes.

4.1.4. Männikjärve drained forest (Estonia)

The site is a Scots pine dominated forest grown on a terrain formerly belonging to Männikjärve bog. In the surroundings of the bog, a drainage ditch system was established in the 1950s (Veber 1974) and was reconstructed in 1975. The drainage lowered the peatland water table from an annual depth of 15 cm to 38 cm below soil surface. Substantial lowering of the water table resulted in significantly improved tree growth (Niinemets *et al.* 2001; Portsmuth *et al.* 2005) and forest density. Therefore, when this study took place, the Scots pine stand was 10–12 m tall in the drained part of the bog compared to 1–3 m in the non-drained part.

4.1.5. Speuldebos (The Netherlands)

The Speulderbos site (Su *et al.* 2009), operated by the National Institute for Public Health and the Environment (RIVM), is located in the central Netherlands. It is approximately 25 km northeast from the city of Amersfoort, within a large forested area in the Netherlands. The site supports a dense 2.5 ha Douglas fir (*Pseudotsuga menziesii* (Mirb.)

Franco) stand. The site topography is slightly undulating with height variations of 10 to 20 m within distances of 1 km.

4.1.6. Los Alcornocales (Spain)

The study sites in Los Alcornocales (North and South plots) (Morillas *et al.* 2012) are situated in a Natural Park in southern Spain, near to the Strait of Gibraltar. The dominant climate is Mediterranean, but the strong maritime influence and the elevation of the terrain enhance its precipitation regime and keep the air temperature relatively mild all year long. There are four plots in the northern part of the Natural Park, and four plots in the southern part. Each plot included five cork oak trees (*Quercus suber* L.), thus 40 trees in total were studied. Due to the proximity of the south plots to a large industrial area (including coal-burning power plants), the south plots are influenced by N and S atmospheric deposition. In the South plots, there is a forest dieback problem, possibly related to side effects of the industrial power plant.

4.2. Leaf nitrogen retranslocation during senescence

At Los Alcornocales (**Paper I**), the N and P resorption efficiencies were calculated as the difference between the annual maximum leaf nutrient concentration in green leaves and the leaf nutrient concentration in senescent leaves and expressed as percentage of the green leaf concentration per plot. The N and P resorption efficiencies were calculated per unit of leaf surface to minimize the effect of mass loss during senescence (Poorter *et al.* 2009).

The nutrient resorption proficiency (NRP) was defined as the minimum N and P concentration per unit of leaf mass found in senescent leaves (Killingbeck 1996). Values of above 1 % and 0.05 %, respectively, were considered to represent incomplete resorption. Complete resorption was defined as a NRP value below 0.7 % for N and 0.04 % for P (Killingbeck 1996).

At Hyytiälä and Speulderbos (**Paper II**), as the litterfall was sampled more exhaustive and there was a possibility to calculate the nitrogen resorption rate (NRR) as the increment of N concentration per day (Δ $\mu g\ g^{-1}\ day^{-1}$). It was calculated after a linear regression of the senescence period.

Annual N resorption efficiency (NRE) (kg N m^{-2} yr^{-1}) in Hyytiälä from Scots pine was calculated as shown in equation 1:

$$NRE = 1.53\ m_b c_g \times m_b c_b \quad (1)$$

where m_b is the mass of brown needle litter collected per square meter during one year, c_b is the N concentration of brown needle litter, c_g is the N concentration of N green needle (1.2 %; Palmroth and Hari 2001). 1.53 is the relative mass of a single green needle to a single brown needle, calculated as an average of all data from unfertilized sapling and mature plots presented by Helmisaari (1992).

In Speulderbos, the NRE and NRR in Douglas fir were calculated from the different concentrations between maximum annual green needle N concentration and minimum annual brown needle N concentration (NRP).

The higher the NRE level and the lower the NRP value, the better the efficiency of use of the nitrogen element in the plant. This usually means that the tree is in a N availability deficiency situation: the plant must prioritize the re-use of the internal elements from the photosynthetically active leaves before litterfall.

4.3. Litterfall collection

Tree litter was collected with litter traps in Hyytiälä and Speulderbos (**Paper II**). Litter traps (mesh size < 0.5 mm) were placed above the topsoil. Twenty circular litter traps of 0.2 m^2 in Hyytiälä (Ilvesniemi *et al.* 2009) and 10 squared litter traps of 0.25 m^2 in Speulderbos were used. Distance between traps was 10 m. Tree litter was collected every ca. 30 days. Litter from each trap was enclosed in labeled plastic bags and transported to the lab. Samples were first air-dried at room temperature until constant mass. In Hyytiälä, during the monthly sampling in winter time, snow was carefully removed from the collectors so that snow did not flow over from the collectors. During winter, samples with snow cover were melted indoors.

Litter was sorted manually with tweezers into four groups: needles, twigs (diameter < 1 cm), bark, and cones; the sum of these groups is what

we considered *canopy fine litter*. When litter fragments were difficult to identify due to their small size, they were considered the rest fraction. The rest fraction mainly consists of brittled twig bark and small needle pieces, including sometimes resin droplets. Twigs which diameter was > 1 cm where not considered fine canopy litter, thus discarded in the total canopy litter values. After sorting, the mass of different litter fractions was determined after oven-drying at 60 °C to constant mass. Only the litter from the dominant conifer species (Scots pine in Hyytiälä and Douglas fir in Speulderbos) was considered.

At Järvselja (**Paper III**), tree litterfall was collected in litter traps for measuring the volatile organic compound emission. One-square meter litter traps were installed in three types of stands dominated by the following species: (1) Norway spruce, (2) Scots pine, and (3) Silver birch and Downy birch. The litterfall collected, despite being dominated by one or other species, presented a more rich inventory of tree species than Hyytiälä, Speulderbos and Los Alcornocales. The litterfall was representative of the main mixed forests growing under hemiboreal climate in Estonia.

At Los Alcornocales (**Paper I**), on the May sampling date (coinciding with the leaf-fall peak), additional senescent leaves were collected by gently shaking the tree branches. Soil and leaf samples were transported in polyethylene bags in coolers to the laboratory. Samples were stored at 3 °C in laboratory refrigerators and processed as soon as possible (less than a week in all cases).

4.4. Leaf N and P content

Total N content of oven-dried leaf samples from Hyytiälä and Speulderbos (**Paper II**) were determined by dry combustion method. Samples from Hyytiälä during 1999-2005 were analyzed by an elemental CN analyzer (LECO, Leco Corporation, St. Joseph, MI, USA). Samples from Hyytiää during 2006-2010 and from Speulderbos were analyzed by a Vario MAX CNS elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

Leaf samples from Los Alcornocales (**Paper I**) were oven-dried at 80 °C until constant weight. Later, the leaf samples were milled to pass through

a 2-mm mesh screen and stored for subsequent N and P chemical analysis. The leaf samples were digested with a sulfuric peroxide mix following Allen *et al.* (1986). The N and P concentration in the digested aliquots were estimated by colorimetric methods (indophenol blue for N and molybdenum blue for P) by using a microplate reader Jupiter G019060 (Asys Hitech GmbH, *Eugendorf, Austria*) (Sims *et al.* 1995).

4.5. Volatile organic compounds emission

At Järvselja, volatile organic compound emission from tree litter was measured (**Paper III**). Litter from three differently dominated mixed forest stands was used. The experimental setup for the litter VOC emission measurements in laboratory conditions is explained in Figure 2.

A subsample (ca. 15 g) of fresh litter was taken from the litter traps for the measurement. The VOCs were adsorbed onto multibed stainless steel cartridges (10.5 cm length, 3 cm inner diameter, Supelco, Bellefonte, USA) filled with Carbotrap C 20/40 mesh (0.2 g), Carbopack C 40/60 mesh (0.1 g) and Carbotrap X™ 20/40 mesh (0.1 g) adsorbents (Supelco, Bellefonte, USA) at a flow rate of 200 mL min^{-1} for 20 min (altogether 4 L air). Background air samples were collected from the empty chamber before and after the measurements. The adsorbent cartridges were analyzed with a combined ShimadzuTD20 automated cartridge desorber and Shimadzu QP2010 plus GC-MS instrument (Shimadzu Corporation, Kyoto, Japan). The TD20 parameters, GC-MS conditions and compounds identification were presented in detail in (Toome *et al.* 2010). Fifteen terpenous compounds were quantified.

At field experiments in Järvselja (**Paper IV**), leaf volatiles organic compounds (VOC) emission was measured by diverting a part of the Walz leaf and conifer cuvette outflow (Walz, GFS3000, Walz GmbH, Effeltrich, Germany) onto adsorbent cartridges. The procedure of analysis was similar to the described above with a flow rate of 250 mL min^{-1} for 10 min (altogether 2.5 L air).

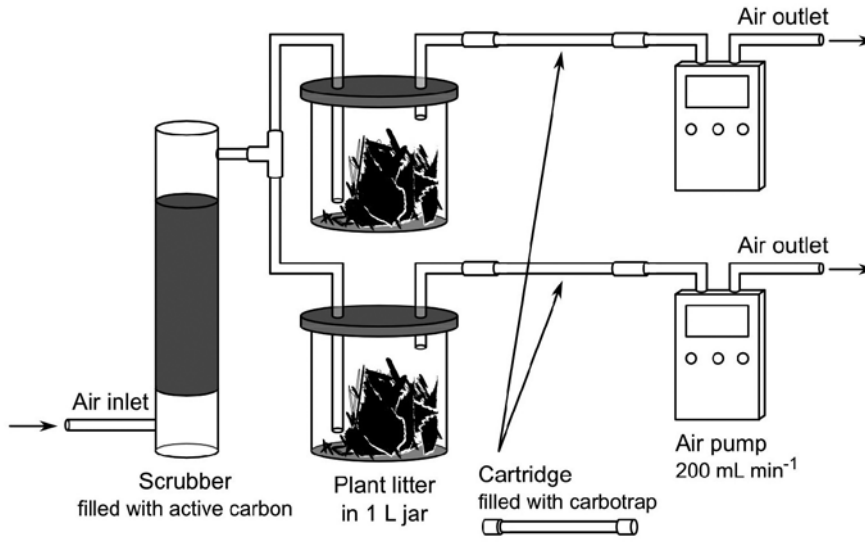

Figure 2. Experimental setup for the litter VOC emission measurement in laboratory.

4.6. Manual soil chamber measurements for soil volatile organic compounds and greenhouse gas emission

At Männikjärve bog (**Paper V**), Männikjärve drained forest, and Järvselja, circular stainless steel collars with a diameter of 305 mm were inserted about 6 cm into the top soil. The placement of the collars took place in the beginning of the campaign in 2008 and measurements conducted refer to the campaign in 2009 (Figure 3). The effective chamber volume (23.8 L) of the cylindrical stainless steel chambers was calculated from the average height from the soil surface to the top of the chamber. The chamber atmosphere was mixed by a small built-in fan to prevent the formation of gradients (Frenzel and Karofeld 2000) and sealed air tight with water. Pre-installed thermocouple sensors connected to a thermocouple reader (Comark KM330) registered the temperatures in soil (–0.1 m, –0.15 m, –0.25 m of depth), inside the chambers, and in the atmospheric air (0 m, 0.5 m, 2 m of height).

Samples were drawn into 100 mL gas-tight syringes from the top of the chamber via a capillary (diameter 2 mm). Thirty seconds before each

sampling, the capillary air was mixed for four times using the syringe. The samples were injected into 7 mL non-evacuated vials (Labco Exetainer 768W). The vials were flushed with 80 mL of sample air and over-pressurized with 20 mL of sample air. The chambers were operated in closed static mode and closed for a maximum of 30 min. 6–10 samples were collected during this time. These were stored in darkness at –18 °C and analyzed within 2 months after sampling by gas chromatography with a GC-FID/ECD (model HP 6890) and a GC-TCD (model HP 7890) as described by Syväsalo *et al.* (2004). All flux rates were calculated from the exponential change in gas concentrations in the chamber and temperature corrected (Noe *et al.* 2011; Appendix A). Air temperature inside the chambers was taken each time a sampling was conducted.

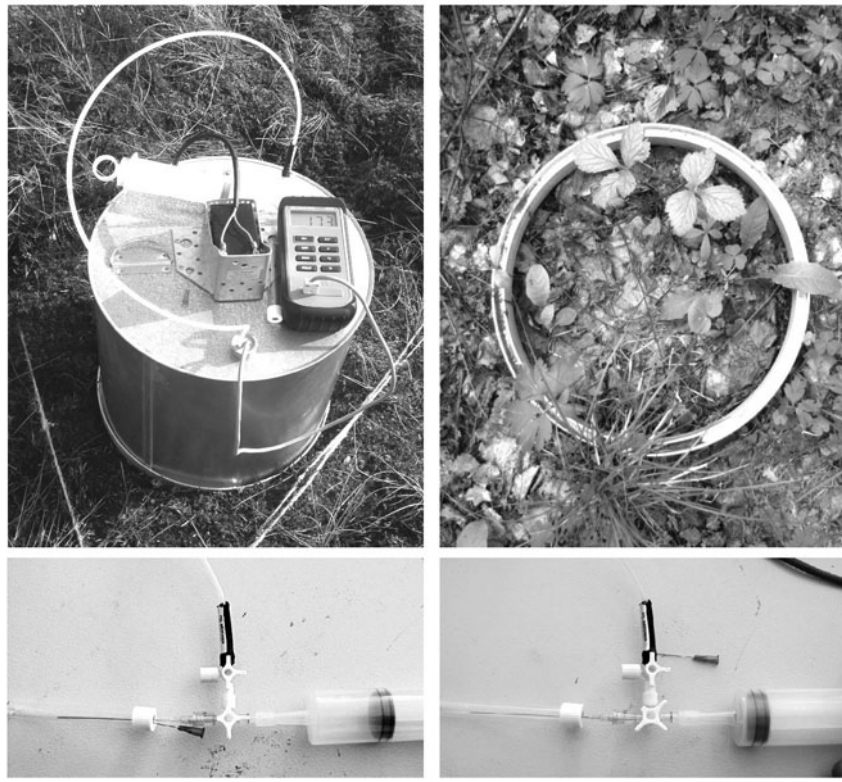

Figure 3. Soil chamber during soil GHG fluxes measurement at Männikjärve (up left). Soil collar installed at Järvselja (up right). Down, the setup for driving the gas sample from the soil chamber to the sampling vial: during the injection of the air sample into the vial by flushing the sample (down left), and after the over-pressurization of the sampling vial (down right), keeping the high pressure inside the vial by closing the T-piece.

At Järvselja (**Paper IV**), VOC emitted from soil were measured using the manually operated soil chambers. The sampling lasted 20 min at a 250 mL min^{-1} flow rate. After sampling a background air sample, the chamber was closed for a period of 30 min and then the sample was taken onto the steel cartridge. To avoid under-pressure in the water-sealed chamber, we allowed background air to enter via a small Teflon tube to the bottom of the chamber during the sampling using a valve sealing that allows to open an inlet while sampling. That led to a change in the operation mode from a closed static chamber to an open dynamic chamber. The data handling is described in detail in (Noe *et al.* 2011; Appendix A). Subsequent GC-MS analysis of the VOC soil emissions have been conducted as described in section 4.4.

4.7. Soil sampling and analysis

At Männikjärve soil (**Paper V**), soil samples were collected in the upper soil layer (O-horizon). The samples were extracted with 2M KCl to measure concentrations of NH^{+}–N and NO_3^{-}–N by colorimetric assays.

At Los Alcornocales (**Paper I**), three soil samples were taken from the top 10 cm of the soil profile and combined to form one composite sample per tree (40 composite samples in total per sampling). The soil samples were oven-dried (80 °C), milled and sieved to pass through a 2-mm mesh screen. The total soil N was measured by standard Kjeldahl procedures (Rutherford *et al.* 2007). To measure soil N, soil subsamples (20 g) were extracted with 100 mL of 0.5 M K_2SO_4 and shaken for 1 h. The extract was filtered with a 0.45-µm Millipore filter. The NH_4^{+}–N and NO_3^{-}–N levels in the extracts were measured by colorimetric method (indophenol blue) using a microplate reader (Sims *et al.* 1995). The soil PO_4^{3-}–P was extracted with 100 mL of 2.5 per cent CH_3CO_2H, and the concentration in the extract was determined by the molybdenum blue colorimetric method (Allen *et al.* 1986). For acid soils, this extraction is equivalent to the standard 0.5 M sodium bicarbonate method used in neutral and basic soils (Allen *et al.* 1986; Hedley *et al.* 1982).

4.8. Numerical analyses

In **Paper II**, data of litterfall and meteorological parameters were calculated by regular periods of one calendar month each: the dates of litter collection period did not correspond to calendar months, therefore, the value of daily litterfall rate measured at the end of each period was considered the same for every day of the collection period; an average daily value was then calculated for each calendar month as the arithmetic mean of all daily values for the month considered, thus 12 values per year. This transformation resulted in the standardization of number of data points along the year, and eliminated differences between both species in the litter collection dates. It permitted to compare statistically both datasets, to make year-to-year comparisons, and improved visual understanding of graphs.

Carbon and nitrogen production rate in litterfall (g C and mg N m^{-2} day^{-1}) were calculated by multiplying the C or N content (g C and mg N g^{-1} biomass) of each litter fraction for a given collection period by the respective litterfall rate (g m^{-2} day^{-1}). The values of C and N production rates for each collection period were subsequently transformed to calendar monthly values as explained above, thus obtaining one value per calendar month.

For Hyytiälä site, the identification of two seasons in monthly C and N input to the forest floor was found after a paired t-test: monthly values during the study period were grouped into two seasons (colder season vs. warmer season, covering the whole year period) throughout the possibilities (varying the season length and the starting month), the option which produced the highest significance (lowest P value) in a t-tests was chosen as the most appropriate way to study the two seasons in each study site.

5. RESULTS

5.1. Nitrogen deposition and leaf N content

The sites of study were differently influenced by N deposition. After Fagerli *et al.* (2005) (Figure 4), the exceedance of nutrients on ecosystems in 2003 was < 200 eq ha^{-1} yr^{-1} in Hyytiälä, Männikjärve and Järvselja; from 400 to 600 eq ha^{-1} yr^{-1} in the north plots of Los Alcornocales; and > 800 eq ha^{-1} yr^{-1} in Speulderbos and the south plots of Los Alcornocales.

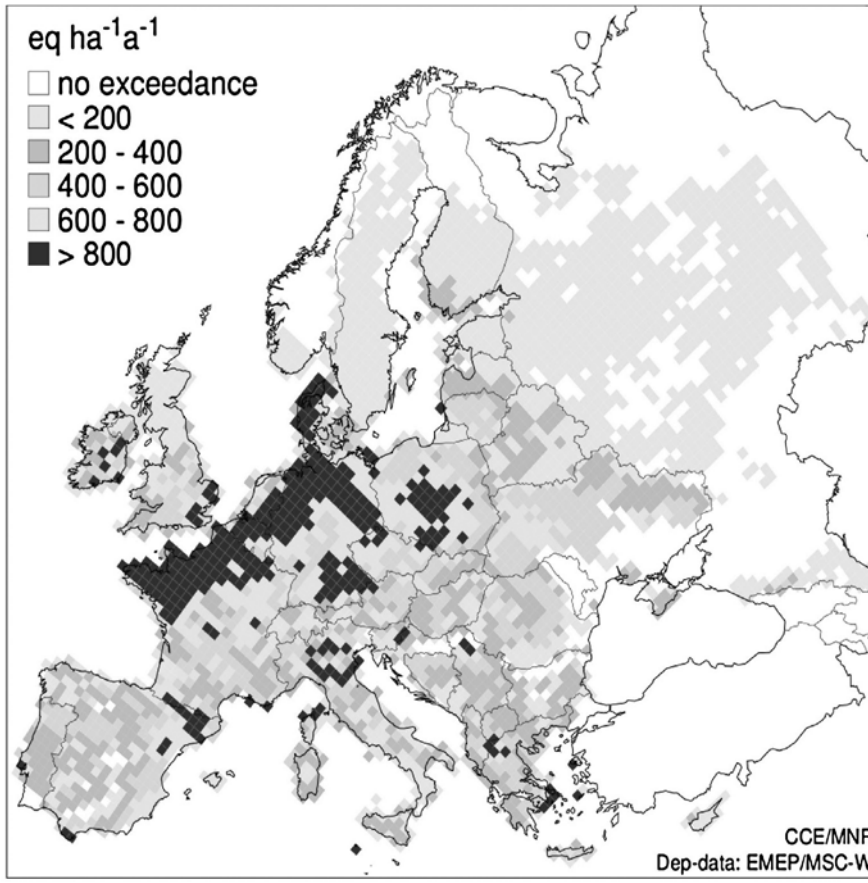

Figure 4. Load of excess nutrients in Europe in 2003 (Fagerli *et al.* 2005).

At Hyytiälä, the site with lowest N deposition (Figure 4), the Scots pine trees produced leaf litter with a N content of 0.663 ± 0.038 %. These values are about 2.8 times smaller than for Douglas fir leaf litter (1.80 ± 0.23 %) produced in Speulderbos, highly impacted by N deposition (Figure 4).

At Los Alcornocales, the contrasting N deposition rates affected to the leaf N content by increasing its value in the trees under higher N deposition. The leaves sampled before senescence period (February) in the South plots showed higher N content (1.86 ± 0.06 %) than the leaves from the North plots (1.57 ± 0.07 %). Leaf N concentrations between North and South plots were significantly different throughout the year ($F = 6.15$, $df = 31$, $P = 0.019$).

At Los Alcornocales, the mean soil NH_4^+–N, NO_3^-–N and PO_4^{3-}–P values (μg g^{-1}) were low in both the South and the North plots (Table 2), but significantly higher values were found in the North plots for soil NO_3^-–N. Despite the different N deposition rates between the North and South plots, no other significant differences in soil properties were found between the South and North plots.

Table 2. Soil chemical properties at two sites (four plots in each site) at Los Alcornocales Natural Park (modified from **Paper I**).

Soil trait	South plots	North plots
pH	5.05 ± 0.40	5.81 ± 0.31
Water content	15.6 ± 1.8	15.0 ± 1.8
Acetic acid PO_4^{3-}–P (μg g^{-1})	1.43 ± 0.14	2.25 ± 0.32
NH_4^+–N (μg g^{-1})	4.9 ± 0.6	3.93 ± 0.43
NO_3^-–N (μg g^{-1})	2.05 ± 0.41	3.6 ± 0.9
Total N (%)	0.169 ± 0.008	0.164 ± 0.008
C/N	7.99 ± 0.14	8.29 ± 0.46

5.2. Leaf senescence and nitrogen resorption

In Hyytiälä and Speulderbos, the nitrogen resorption traits were calculated from long-term litterfall data collected for the **Paper II**. The period of N resorption, counting from the senescent leaves maximum concentration to the lowest, was different in each year in Scots pine (Hyytiälä), and more variable than in Douglas fir (Speulderbos). NRP values in Scots pine were significantly lower than for Douglas fir in Speulderbos ($P < 0.001$). N resorption rate (NRR) of senescent foliage was slower in Scots pine (Hyytiälä) than in Douglas fir (Speulderbos).

Green leaf N concentration correlated negatively with leaf litter N concentration produced four months later ($r^2 = 0.58$, $P < 0.001$) in Scots pine (Hyytiälä). Annual average values of NRE versus NRP for the period 1999-2009 were negatively correlated for Scots pine ($r^2 = 0.90$, $P < 0.001$).

The average nitrogen resorption efficiency (NRE) in Scots pine (Hyytiälä) was 64 %, and 33 % for Douglas fir (Speulderbos). Average nitrogen resorption proficiency was 0.52 % for Scots pine and 1.52 % for Douglas fir.

At Los Alcornocales (**Paper I**), the N resorption proficiency values indicated incomplete resorption for most cork oak individuals in both the North and the South plots (Figure 5c). However, the P resorption proficiency values showed a complete resorption for all 40 oak individuals (Figure 5d). Interestingly, P resorption efficiency rates were very high (Figure 5b). No significant differences between the North and the South oak populations were found for N ($F = 1.5$, $df = 1$, $P = 0.266$) or P ($F = 0.41$, $df = 1$, $P = 0.547$) resorption proficiencies (Figure 5a, b), despite the different N deposition rates.

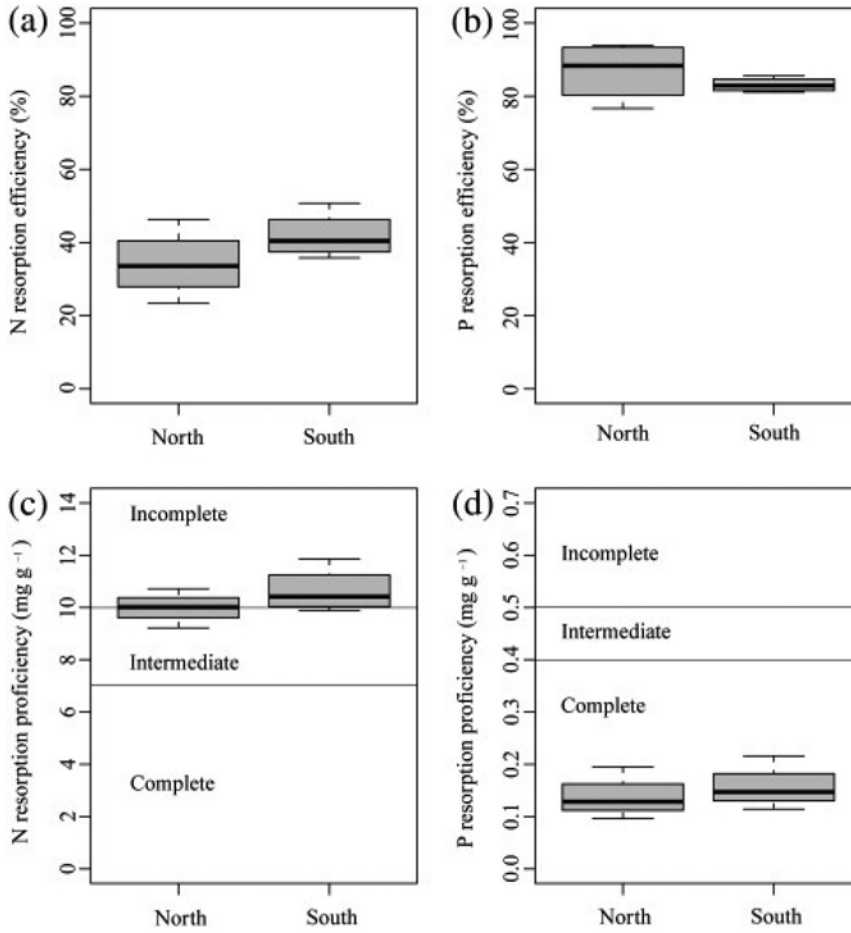

Figure 5. (a) N resorption efficiency (calculated per unit of leaf surface) in the South and the North cork oak plots. (b) P resorption efficiency (calculated per unit of leaf surface) in the South and the North cork oak plots. (c) N resorption proficiency in the South and the North cork oak plots. (d) P resorption proficiency in the South and the North cork oak plots. The figure shows the median and the 10th, 25th, 75th and 90th percentiles. The horizontal lines show the threshold values of resorption proficiency, following Killingbeck (1996) (**Paper I**).

5.3. Litterfall dynamics and quality

The average (± SE) annual fine litter dry mass production over the study period was 2.86 ± 0.22 Mg ha^{-1} yr^{-1} for the Scots pine stand in Hyytiälä (1999-2011) and 4.34 ± 0.33 Mg ha^{-1} yr^{-1} for the Douglas fir stand in Speulderbos (2008-2010). Fine litter production rates in Scots pine showed an unimodal distribution with a maximum in autumn (2.82 g m^{-2} day^{-1} in August 2006, 1.43 in September 2007, 3.27 in October 2008, 2.84 in September 2009, and 1.99 in September 2010) (Fig. 6a).

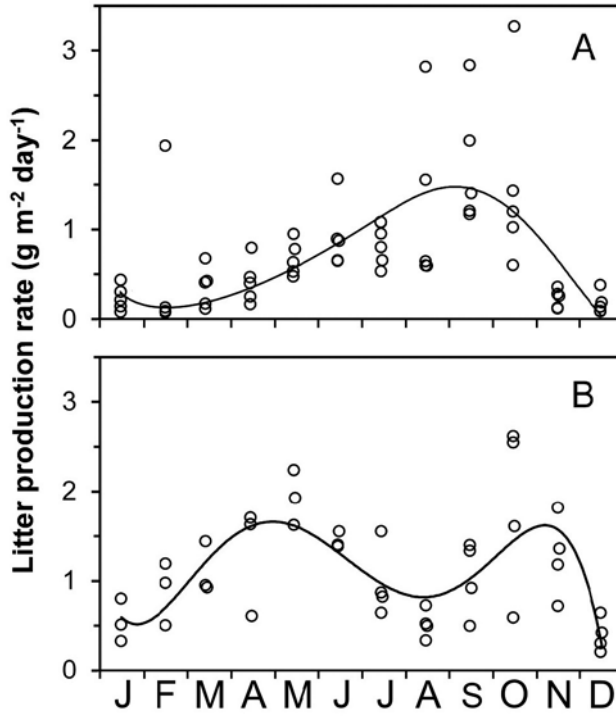

Figure 6. Monthly average litter production rate in Hyytiälä (a) for 2006–2010 and Speulderbos (b) for July 2007 to December 2010. The trendlines correspond to third-order (a) and fifth-order (b) polynomial regressions with r^2 values of 0.46 (a) and 0.47 (b) (P<0.001 for both)(modified from **Paper II**).

During snow cover period (November-March) a small amount of litter was produced. Minimum Scots pine litterfall rates were reached in months between December to February, and ranged from 0.078 to 0.127 g m^{-2} day^{-1}. Douglas fir (Fig. 6b) showed a bimodal distribution. Litterfall was more evenly distributed during the year, and maximum peaks were less pronounced than for Scots pine because of the less severe winter period in Speulderbos. Nevertheless, litter was mainly produced from April to May and from October to November. Annual maximum values of litter production were 1.93 and 1.61 g m^{-2} day^{-1} in May and October 2008, 2.24 and 2.54 g m^{-2} day^{-1} in May and October 2009, 1.71 and 2.62 g m^{-2} day^{-1} in April and October 2010 for these two peaks (Fig. 6b). Annual minimum values were reached in December and January, with values from 0.21 to 0.33 g m^{-2} day^{-1}.

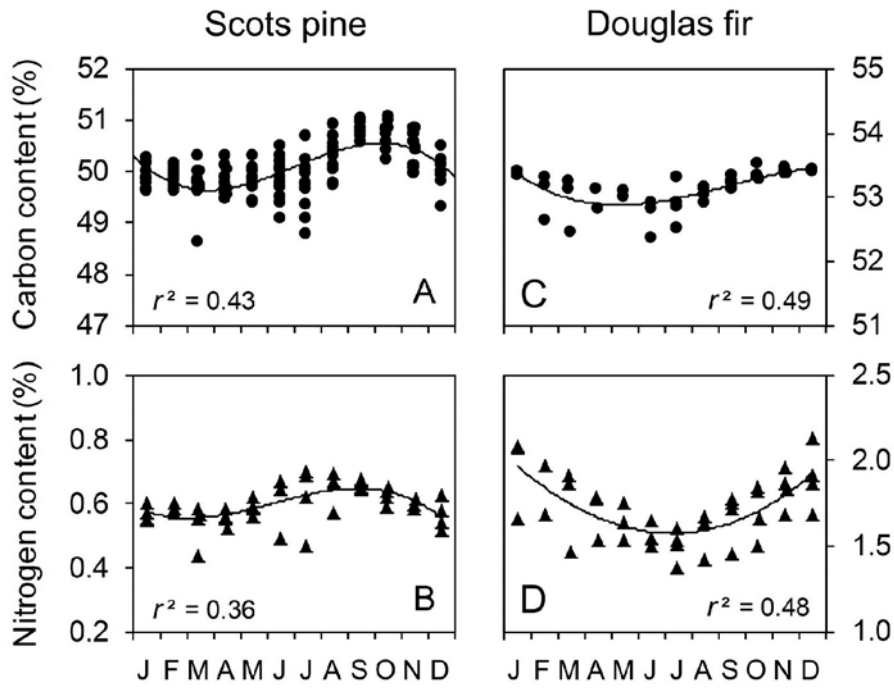

Figure 7. Litterfall N content of Scots pine (1999–2009) in Hyytiälä and Douglas fir (July 2007 to December 2010) in Speulderbos. Data correspond to monthly averages during the study period. Data were fitted by 3rd-order polynomial regressions; both are significant at $P < 0.001$. (Modified from **Paper II**).

Annual average litter N concentration in Scots pine (Hyytiälä) was 0.601 ± 0.036 % (Figure 7b), in Douglas fir (Speulderbos) was 1.7 ± 0.5 %, and in Järvselja 1.53 ± 0.10 % (Figure 7d). Litter C concentrations were 50.27 ± 0.24 %, 53.1 ±1.1 % (Figure 7a), 48.65 ± 0.72 % (Figure 7c), respectively. From a temporal view, litter N content fluctuated more along the year in Douglas fir than in Scots pine.

The litter C and N content did not show any significant Pearson's linear relationship with the litterfall rate in any of both species ($r^2 < 0.012$). But the litterfall C and N input to soil rates were highly correlated between each other, and with the total litterfall and needle litterfall (Table 3). Other interesting linear models between total litterfall, needle litterfall and C and N inputs to soil from litterfall are showed in Table 3.

Table 3. Equations of linear models ($y = ax + b$) fitted to litterfall characteristics relationships: y correspond to the traits in the second column, x correspond to the titles of the following columns. Scots pine (Jan 2006 – Dec 2010) and Douglas fir (Jul 2007 – Dec 2010).

Species	Litter traits	LF_{tot}	LF_{nee}	LF_C
Scots pine	LF_{nee}	$0.8895x - 0.2023$		
	LF_C	$0.5094x - 0.0042$	$0.5190x + 0.1355$	
	LF_N	$0.0057x - 0.0002$	$0.0061x + 0.0012$	$0.0113x - 0.0002$
Douglas fir	LF_{nee}	$0.8195x - 0.0360$		
	LF_C	$0.5325x + 0.0001$	$0.6035x + 0.0632$	
	LF_N	$0.0176x - 0.0003$	$0.0200x + 0.0018$	$0.0328x - 0.0002$

LF_{tot} and LF_{nee} are total litterfall and needle litterfall rates (g day^{-1}); LF_C and LF_N are carbon and nitrogen production rates (g m^{-2} day^{-1}).

Parameters are monthly-averaged. The linear regressions are calculated with monthly values (n = 60 for Scots pine; n = 42 for Douglas fir).

Pearson's linear correlation coefficients (r^2) are from 0.839 to 0.999, all coefficients are significant at $P < 0.001$ (two-tailed).

5.4. C and N input rates to soil

In Hyytiälä and Speulderbos, annual C and N inputs to soil were mainly driven by needle litterfall. The annual N input to soil through litterfall was 15.9 ± 1.5 kg N ha^{-1} yr^{-1} in Hyytiälä and was much lower than in the Speulderbos stand (75 ± 8 kg N ha^{-1} yr^{-1}). If comparing the C annual input between Hyytiälä (1430 ± 110 kg C ha^{-1} yr^{-1}) and Speulderbos (2300 ± 180 kg C ha^{-1} yr^{-1}), the same pattern was revealed (**Paper II**).

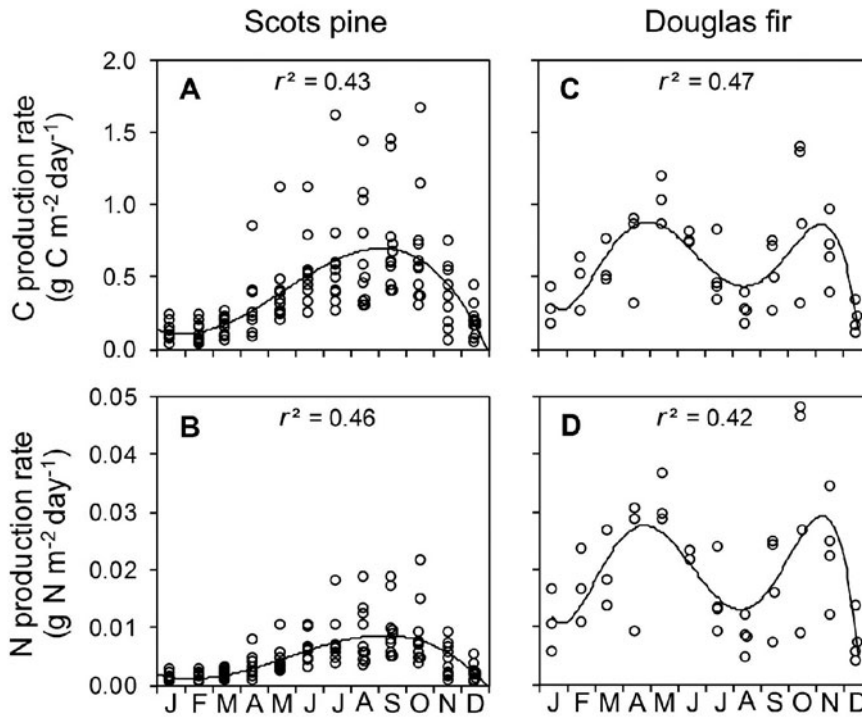

Figure 8. Carbon and nitrogen production rate in litterfall of Scots pine (1999-2009) in Hyytiälä and Douglas fir (July 2007 to December 2010) in Speulderbos. Data of Scots pine were fitted by 3rd order polynomial regression and those in Douglas fir by 5th order polynomial regressions. (**Paper II**).

In Hyytiälä were two different seasons regarding the C and N inputs to soil through litterfall detectable: May–October and November–April (paired-samples *t*-test, $P < 0.001$). Average (± SE) C input rates to soil in Hyytiälä were 0.574 ± 0.039 g C m^{-2} day^{-1} from May to October and 0.204 ± 0.020 g C m^{-2} day^{-1} from November to April. N input rates for these seasons were 6.48 ± 0.49 and 1.969 ± 0.037 mg N m^{-2} day^{-1}, respectively.

Generally, there were two periods during the year at the Douglas fir site when C and N were released to the topsoil from the canopy litter (Figure 8c, d), while there was only one period at Hyytiälä site (Figure 8a, b).

5.5. Monoterpene emissions from foliage, litter layer and soil at Järvselja

Largest monoterpene emission rates (sum of monoterpenes, 46 ± 9 nmol m^{-2} s^{-1}) were found from Silver birch with α-pinene as the major monoterpene emitted. Norway spruce emitted a total monoterpene flux of 33 ± 11 nmol m^{-2} s^{-1}, where delta-3-carene was the main contributing monoterpene followed by α-pinene and α-phellandrene (Figure 9). The isoprene fluxes for *Silver birch* (1.2 ± 0.6 nmol m^{-2} s^{-1}) and *Norway spruce* (1.02 ± 0.36 nmol m^{-2} s^{-1}) showed no substantial differences. Compared to the total monoterpene emissions, isoprene emissions were about 45 times smaller.

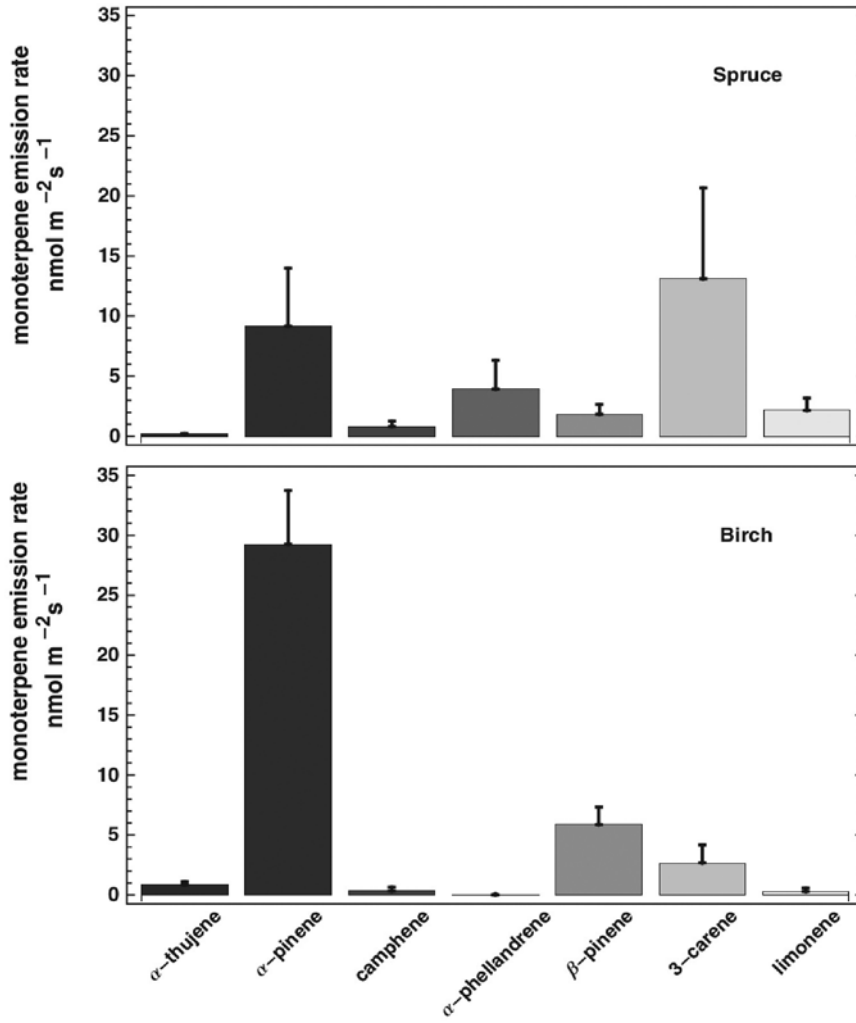

Figure 9. Monoterpene emissions from the main tree species Silver birch and Norway spruce, averaged over leaves and branches differently exposed to sunlight. West, South and North exposed leaves or branches. Error bars indicate standard errors (adapted from **Paper IV**).

Monoterpene emission rate from soil was 40 times smaller (1.22 ± 0.16 nmol m^{-2} s^{-1}) than green leaf emission rate. α-phellandrene was lacking completely from soil VOC emissions (Figure 10). The compounds α-thujene and limonene, which were less abundant at the leaf emission level were main contributors to the soil monoterpene fluxes.

In Järvselja (**Paper III**), seasonal differences in the total emission of litter VOCs were found, defined by a maximum in summer and a minimum in autumn and winter. During summer months, litter emissions were dominated by limonene, α-pinene, camphene and 3-carene in the three litter types, accounting for 70-75 % of total VOC emitted in June. 3-carene, α-pinene and β-pinene were the main compounds emitted during winter time, accounting for 50-60 % of total VOC emitted in January.

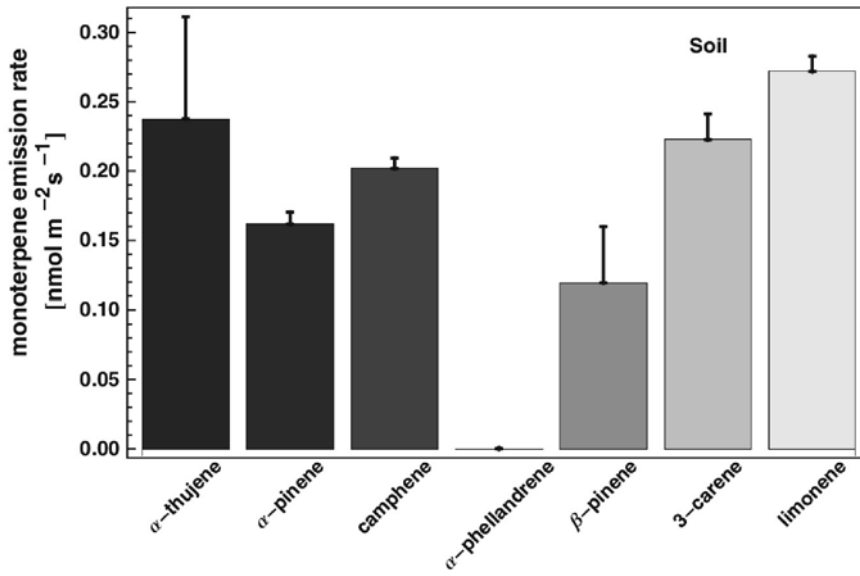

Figure 10. Monoterpene emissions from soil and leaf litter. Error bars indicate standard errors.(adapted from **Paper IV**).

5.6. Soil GHG emissions in three stages of peatland drainage

The soil volatile organic compounds emissions was measured in three sites: Männikjärve bog, Männikjärve drained forest, and Järvselja. The sites represented three stages of the peatland drainage practice, where the water table is lowered, thus the soil water content. The three sites presented an annual average water content of 77, 37, and ca. 23 % (vol/vol), respectively. The water table was lowered from 15 cm to 38 cm in Männikjärve, and it was 45 cm in Järvselja. The soil GHG compounds measured were the CH_4, N_2O and CO_2.

At Männikjärve, there were substantial differences between the GHG fluxes measured along the year in the two plots ($P < 0.001$) (Table 4).

Table 4. Annual cumulative GHG soil fluxes at Männikjärve (mean of 3 replicates ± SE).

Männikjärve	mg CH_4–C m^{-2} yr^{-1}	mg N_2O–N m^{-2} yr^{-1}	g CO_2–C m^{-2} yr^{-1}
Bog	2729 ± 180	41.8 ± 5.7	528 ± 66
Drained forest	943 ± 222	4.1 ± 7.9	226 ± 56

In Järvselja (**Paper IV**), negative CH_4 fluxes were found during the summer campaign in 2009 (daytime average CH_4 flux was –0.69 ± 0.36 nmol m^{-2} s^{-1}), which indicated a possible net consumption of CH_4 in soil. Daytime N_2O emissions averaged 0.18 ± 0.09 nmol m^{-2} s^{-1}.

6. DISCUSSION

6.1. N deposition, leaf N content and litter N content

At Los Alcornocales (**Paper I**), leaf N concentration in the North plots (low affected by N deposition, Figure 4) were similar to values reported by Ramírez-Valiente et al (2011) at La Almoraima (Los Alcornocales Natural Park), Alcácer do Sal (central Portugal) and Aïn Rami (northern Morocco). The cork oak trees exhibited higher leaf N concentrations and higher leaf N:P ratios in the South (high-mortality plots) plots than in the North plots. The higher leaf N concentration in the high mortality plots could be related to the proximity of fossil-fuel power plants, which have been responsible for the high N deposition rates detected over the past 30 years. An increase in leaf N concentrations and nitrate assimilation is one of the symptoms of N saturation (Aber and Melillo 2001; Lovett and Goodale 2011; Tessier and Raynal 2003). However, the measurements of soil N and P extractable pools could be biased by the lower tree density in the high mortality plots. It might have favored the leaching and denitrification of NO_3^-–N from soil. Such losses of N occur in response to ecosystem disturbances that disrupt the biological mechanisms of NO_3^-–N retention (Vitousek and Howarth 1991). Furthermore, the soil N and P pools showed high spatial and temporal variability (Rodríguez *et al.* 2009). In conclusion, at Los Alcornocales, the extractable soil nutrient pools appeared to be a weak estimate of nutrient availability, and the measurement of mineralization or nitrification rates may be a more adequate and conclusive method to demonstrate differences between sites of different N deposition inputs.

The N deposition is higher in Speulderbos than in Hyytiälä (Figure 4). Speulderbos employed a higher leaf litter N content as compared to Hyytiälä (Figure 7), which was confirmed by Wang *et al.* (2013). This suggested that the litter N content could be related to the soil nutritional status, despite differences in tree species' physiology and different climatic conditions. The higher fertility of the Speulderbos site might be related to higher litter N content (Wang *et al.* 2013).

Wang and colleagues reported similar values of green leaf annual N content in our same experimental sites: Scots pine's ranged from 1 % to 1.3 %, which was smaller than 1.5 % to 2.2 % in Douglas fir. These results could be possibly related with the lower soil fertility.

Beside the direct effects of N addition on forest ecosystems, it can induce P limitation (Vitousek *et al.* 2010). For example, extraordinarily high levels of atmospheric N deposition in northwestern Europe have removed N limitation at many sites (Berendse *et al.* 1993; Stevens *et al.* 2004; Vitousek *et al.* 2010), and many of the altered systems are now limited primarily by P (Aerts *et al.* 1992; Verhoeven and Schmitz 1991). Speulderbos site could potentially be a place where the N addition would provoke a shift towards limitation of P or other nutrient. The alteration of one of the N flows in the ecosystem cycle (Figure 1) could have affected to the trees' nutritional equilibrium.

6.2. Leaf senescence and nitrogen resorption

Nutrients lost in litterfall are a cost to a tree, as they have to be substituted to maintain primary productivity (Lin *et al.* 2003). Nutrient resorption from senescent leaves can be defined as the mobilization of nutrients from senescent leaves to other longer living structures. It is a conservative way to efficiently recycle the nutrients and minimize the energy costs of nutrient uptake from soil.

According to Killingbeck (1996), the annual minimum of litter N concentration (NRP: nitrogen resorption proficiency) indicated a very good capacity for nutrient resorption in Scots pine (Hyytiälä) (NRP < 0.7% N). In contrast, the annual minimum of Douglas fir litter N concentration was at the level of *incomplete N resorption* (NRP > 1% N) and consequently litterfall rich in nitrogen. In terms of plant nutrition, a high NRP value means inefficiency of use of nutrients. This could suggest that the fertile soils at Speulderbos and the high atmospheric N deposition (Figure 4) might compensate the N loss through litterfall of these trees. Given that idea, this could fit the observed high stand growth rate of 10.7 m^3 ha^{-1} yr^{-1} (Portillo-Estrada *et al.* 2013). But several works concluded that plants growing on low fertile soils do not retranslocate a greater fraction of elements from senescent leaves: resorption efficiency has been found to be independent of the status of individuals (Birk and Vitousek 1986; Chapin and Moilanen 1991; Helmisaari 1992; Schlesinger *et al.* 1989; Walbridge 1991) as well as in **Paper I**.

High nutrient resorption efficiency (NRE) during senescence means that plant is capable to conserve most of the nutrients allocated in leaves,

twigs, etc. before the litterfall. A plant growing on a unfavorable climate or poor soil would need to increase its NRE and lower its NRP values to minimize risks of malnutrition. Our results stated that there were higher NRE values in Scots pine in Hyytiälä (64 %) than in Douglas fir in Speulderbos (33 %). This could suggest a more efficient use of the nitrogen in Hyytiälä, more limited by nitrogen. These values are in concordance with Wang *et al.* (2013), who found NRE values of 37 % in Douglas fir and 71 % in Scots pine in the same stands. However, NRE values in Los Alcornocales were higher under higher N deposition and soil nutrient availability, as found in Nambiar and Fife (1987). The influence of soil N limitation and the soil N excess in relation to the N resorption traits embed needs more research. Specially into the physiological mechanisms triggering the nutrient retranslocation from senescent leaves. Also further effects like a possible shift towards P limitation for some species with N deposition, as discussed by Crowley *et al.* (2012).

As for carbon, it is few times the limiting factor of an ecosystem. Therefore it is considered less impacting than N, P or other scarce elements for the plant nutrition. In respect to carbon resorption, litter carbon concentration also fluctuated during the year, showing a similar pattern to litter N concentration in Scots pine and Douglas fir (Figure 7a, c). Carbon resorbed comes from complex molecules such as starch and proteins in senescent leaves (Niinemets and Tamm 2005). These are broken down into smaller units (simple sugars and amino acids) which are soluble and easily transportable into storage cells elsewhere in the plant. Despite the fact that carbon resorption during senescence is not of major importance for plants because of the higher availability through photosynthesis, it also takes place (Figure 1). Carbon resorption is usually lower than N resorption in relative terms and less studied.

6.3. Litterfall chemistry and dynamics

The results of Scots pine needle N content are in concordance with Ukonmaanaho *et al.* (2008), which reported a mean of 0.49 % in senescent needles in Finnish forests. The narrow fluctuation range and low annual average of Scots pine litter N concentration observed in Figure 7b is typical for a species adapted to N limited ecosystem. These characteristics indicate that tree do not shed litter rich in nitrogen

because its priority is to conserve it in the internal plant nutrient cycle. These values were even lower than the N content reported by Niinemets *et al.* (2001) (0.86 ± 0.12 %) in a low fertile site in Estonia.

Concerning Douglas fir, Turner and Olson (1976) reported a value of 0.57 % for a 42-year-old Western Washington stand. Ranger *et al.* (2003) found litter N concentrations of 0.91-1.38 % in a seven-year study in France, with a seasonal dynamic similar to our study. The values found at Speulderbos were still higher, possibly because of the high N deposition, which could have increased needle N concentration (Turner 1977). Furthermore, Simpson *et al.* (2006) found Speulderbos site to be particularly affected by N deposition, while Hyytiälä belonged to the less affected areas in Europe.

In **Paper II**, Scots pine and Douglas fir litterfall dynamic were different (Figure 6). During winter in Hyytiälä, the needles were in dormant phase, and it is beneficial for the plants to drop the needles right before this phase. Therefore leaf litter production in winter is normally small, and presumably caused mainly by physical damage to the trees. At Speulderbos, which had warmer climate, the rate of litter production processes only decreased during winter (Figure 6).

During the two-year litterfall sampling at Järvselja (**Paper III**), we found that stands with different species composition (mixed evergreen and deciduous species) showed similar dynamics through the year (unpublished data). Scots pines and spruces generated a baseline of litterfall along the year. There was a stand litterfall peak in autumn composed by the deciduous species' litterfall as well as evergreen species annual peak. Overall, the litterfall dynamics regarding the cumulative litterfall were similar in the different stands studied in Järvselja.

At the Mediterranean site of Los Alcornocales (**Paper I**), the litterfall was produced mainly in spring-summer. Coinciding with Andivia (2010), the litterfall from cork oak had two annual maxima: the first and most important was in spring (when the renewal of foliar cover happened). The second, and less important, was around October, when the precipitations after summer drought and the mild temperatures produced a second sprout.

6.4. C and N inputs to soil from litterfall

Similar values for annual Scots pine litter production have been reported (Berg *et al.* 1999; Starr *et al.* 2005) in stands in similar latitude and age than Hyytiälä. In the native environment of Douglas fir on the west coast of North America, McShane *et al.* (1983) found similar annual litterfall values in a stand of similar age. Also Ranger *et al.* (2003) reported similar values for a 40-year stand in France.

In Speulderbos, when the stand was 35 years old, Koopmans *et al.* (1996) calculated a N input to soil of 51 kg N yr^{-1} (ca. 0.014 g N m^{-2} day^{-1}), and our results give an input of 0.019 g N m^{-2} day^{-1}. This increase can be interpreted as a result of the stand growth (Ukonmaanaho *et al.* 2008).

There have been studies investigating the annual litterfall amount as a function of the latitude and stand age in Scots pine (Berg *et al.* 1999) across Europe, concluding that it was possible to explain a major part of the litterfall phenomenon in combination with other factors. This could lead to the idea that a stand in a lower latitude could cycle more nutrients through the ecosystem because of its favorable conditions for life.

6.5. Litter decomposition and nutrients mineralization

There are many studies on litter decomposition and most of them treat leaf litter. But we know relatively little about root decomposition, despite the large proportion of litter production that occurs below ground (Lambers *et al.* 2008) and the rapid turnover of below ground biomass.

Briefly, here I mention some important findings on leaf litter decomposition. It is positively related to the specific leaf area, the ratio of leaf area to dry weight (Garnier *et al.* 2004; Cornelissen *et al.* 1999). It is negatively related to leaf toughness (Gallardo and Merino 1993), leaf life span, digestibility and palatability (Lambers *et al.* 2008), lignin:N ratio, and lignin concentration (Berendse *et al.* 1989; Berg and Staaf 1981; Fox *et al.* 1990). Furthermore, decomposition is also associated to mycorrhizal type (Cornelissen *et al.* 2001; Lambers *et al.* 2008), and the habitat type (deciduous versus evergreen habitat). In peat bogs, like Männikjärve, litter is decomposed very slowly. This is because peat moss species produce phenolic compounds which make the leaf litter more

difficult to decompose. Together with the acidic and anoxic ambient of bog environments (Cornelissen *et al.* 2006; Johnson and Damman 1993), these factors lead to an accumulation of litter in peat bogs, forming deep histosols.

A major reason to be interested into the decomposition process is its close link to nutrient supply for soil organisms and plant nutrient uptake. In most ecosystems > 90% of the N and P supply to plants is provided by the nutrients released during decomposition (Lambers *et al.* 2008). Nitrogen is the nutrient whose release from plant litter is most tightly linked to biomass decomposition rate. Plant litter needs to be physically broken down into smaller pieces to be used by microorganisms, and nitrogen is further bound to big molecules and structures which must be also broken down (Lambers *et al.* 2008).

6.6. VOC emission at leaf, litter and soil levels

At Järvselja (**Paper IV**), the measurements conducted at the leaf and soil levels suggested, that α-thujene and limonene may be stored to greater amounts in the leaf tissues related to their synthesis when still attached to the branches and that α-phellandrene seems not to be stored in substantial amounts. Given the fact that deciduous leaves mostly do not build specialized storage organs we can speculate that soil monoterpene emissions are caused by the coniferous litter (Noe *et al.* 2012). The contribution of the different litter parts (needles, leaves, twigs, cones, etc.) to the litter terpene fluxes remains to be investigated for the measurement site. As it is known that beside the leaf litter soil fungi and roots also emit different volatiles (Leff and Fierer 2008), their contribution to the total emitted soil fluxes presented here have to be assessed in future.

In **Paper III**, stand to stand differences were assessed. The spruce and birch dominated stands showed more similarities in their VOC emission pattern if compared to the Scots pine dominated stand. Together with the 2-year litterfall data, further work in this direction will estimate the annual total VOC emitted by the soil litter layer.

The production of VOC by soil microbes is important for the soil ecology and forest biogeochemistry. However, soil VOC production has received

relatively little attention. The spatial heterogeneity of the below ground microorganisms and the many potential sources for the same compounds, makes it difficult to study. As consequence, there is few knowledge about how the emissions vary across soil types, soil fauna, and litter type (Leff and Fierer 2008). Soil microorganisms can be responsible for important emissions of volatiles to the below canopy atmosphere (Bäck *et al.* 2010). The VOC emission from soil organic layer is tightly related to the litter decomposition processes; and the litter decomposition is related to the litter quality, decomposers and climatic conditions. Therefore, as the VOC emission can qualitatively and quantitatively vary much through ecosystems, further research in all these processes is needed to improve our knowledge on the subject.

6.7. Soil water table and soil GHG emissions

By time, plant litter becomes part of the soil organic matter and minerals through decomposition. A subsequent stage of the litterfall is the soil organic matter content. This constitutes the source of nutrients for soil fauna.

The drainage practice in wetlands is the most significant disturbance factor influencing GHG fluxes in these ecosystems. Drainage causes substantial increase in soil CO_2 efflux and N_2O emission as well as decreasing CH_4 emissions (Soosaar *et al.* 2012). A high water table limits the growth of roots mainly because of poor soil aeration. Lowering the water table improves the soil aeration and is directly related to an enhanced stand growth rate and species richness. This practice has been performed in Estonia during the last Century for silvicultural purposes and peat extractions by building a network of ditches.

Nitrous oxide (N_2O) emissions from ombrotrophic bogs, like Männikjärve (**Paper V**), are relatively insensitive to changes in the water table, as the efflux is probably limited mainly by slow N transformation rates. This is caused by low pH and nutrient availability (Martikainen *et al.* 1993). However, soil N_2O net emissions declined due to long-term drainage, which is in contrast to results from previous drainage experiments in ombrotrophic bogs (Martikainen *et al.* 1993; Regina *et al.* 1996). Apparently, increased tree growth at the drained area reduced the availability of soil nitrate (from 2.55 to 1.28 mg NO_3^--N L^{-1}), which

in combination with lower soil moisture caused the decline in N_2O emissions. Nitrate availability seemed to be a driver of N_2O emissions during the drainage process.

In the drained area of Männikarve bog, the prolonged drainage manipulation also showed strong effects on soil respiration rates. Drainage most often leads to increased CO_2 emissions in hydromorphic soils, at least in the short-term (Danevčič *et al.* 2010; Jungkunst and Fiedler 2007; Maljanen *et al.* 2010). However, the drainage treatment at Männikjärve bog started more than 30 years ago and currently, the easy degradable fractions of the peat have already been decomposed and carbon cycling in the ecosystem has reached a new balance. Permanent drainage of Männikjärve bog also reduced the CH_4 efflux significantly ($P = 0.003$).

In terms of total climate forcing response, prolonged drought and long-term (> 30 yr) drainage consistently reduced the soils' greenhouse gas emission (Table 4). Thus, the drainage reduced the bog's contribution to global warming. However, the ecosystem's response to drainage presumably increased soil CO_2 emission during the first years, but reduction dominated in the long run.

At Järvselja, (**Paper IV**), the soil respiration dataset was not long enough to make extrapolations to annual GHG emissions. Also, the measurements were done automatically and manually. Manual measurements had a limited number of data points and were conducted only during daytime. Manual and automatic measurements were comparable, therefore they complemented each other (Noe *et al.* 2011, Figure 7).

The CO_2 soil emissions (3.54 ± 0.62 μmol m^{-2} s^{-1}) are comparable to other boreal forest soils emissions during summer (Kolari *et al.* 2006; Rayment and Jarvis 2000) and cool temperate forest soil fluxes (Lee et al 2002, Yuste 2005).

Net methane consumption in boreal forest soils has also been reported by Whalen *et al.* (1992). If compared to boreal soil methane fluxes in Hyytiälä of about –1.4 nmol m^{-2} s^{-1} (Pihlatie *et al.* 2009b) we found a slightly lower sink flux (-0.69 ± 0.36 nmol m^{-2} s^{-1}). The inverse relationship between the CO_2 and CH_4 fluxes may support the idea of

methane consumption by methanotrophic bacteria in the soil as a reason for the flux.

The N_2O soil fluxes measured at Järvselja were about the same compared to soil N_2O fluxes at Hyytiälä of 0.15 nmol m^{-2} s^{-1} (Pihlatie *et al.* 2009a). N_2O is mainly produced by denitrification process in soils. In deeper layers, given wet and anaerobic conditions denitrification may occur frequently (Ball *et al.* 1999; Yamulki and Jarvis 2002). In our groundwater influenced study site, the soil temperature should correlate positively with the N_2O efflux (Schindlbacher 2004). Due to the limited number of measurements we observed only a weak positive correlation.

7. CONCLUSIONS

Across the publications reached during the doctoral studies, I can conclude that studying plant litter was a justified method for assessing flows and pools of the C and N cycles in the ecosystems studied.

Nutrient status of plant litter was the result of the nutrient resorption during senescence. Plant litter reflected the level of resorption efficiency that trees need in order to survive in their specific environment. It included different atmospheric N deposition load, soil N availability and climatic parameters.

Nutrient resorption proficiency was significantly smaller for Scots pine than Douglas fir. This could suggest that on fertile soils like Speulderbos, together with high atmospheric N deposition rates, the incomplete resorption of these trees was compensated.

Higher N soil fertility led to increased C and N turnover rates in the ecosystem C and N cycle (for example: higher stand growth, higher C and N soil inputs from litterfall, higher leaf litter N concentration). Total litterfall, needle litterfall, total C and N inputs to soil from litterfall were highly correlated to each other in both, Scots pine and Douglas fir stands. This leads to a direct link between the C and N pools in the ecosystem. Therefore, an alteration in one pool or flux of the C and N cycles will be transmitted to other pools and fluxes of the ecosystem.

This thesis also made an overview on the role of soil litter layer in the forest VOC emissions. This was compared to direct leaf level and soil level emissions. These make up the major contributors to in-canopy atmospheric VOC concentration. Litter VOC emissions were mainly caused by coniferous litter, because deciduous species do not build specialized storage organs like resin ducts.

Lowering the peatland's water table for long term successfully permitted to increase the species richness and tree density, and it also increased C and N sequestration into tree biomass. These changes led to a new balance between the ecosystem C and N pools.

The long-term drainage declined soil N_2O, CO_2 and CH_4 net emissions substantially. Which means less contribution to global warming. At Järvselja, longer-term (above century) drained soils, together with forest nutrient use and cycling, reached the status of CH_4 sink.

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SUMMARY IN ENGLISH

ON THE RELATIONSHIPS BETWEEN PLANT LITTER AND THE CARBON AND NITROGEN CYCLES IN EUROPEAN FOREST ECOSYSTEMS

Introduction

Carbon (C) and nitrogen (N) are important macronutrients in the biosphere, and fulfill very different functions in ecosystems. Studies in biogeochemistry focus on the nutrient cycling between the organic and inorganic states of the elements, and the speed of the processes. Molecular nitrogen (N_2) is abundant in atmosphere but the N_2 fixation is crucial for the N input to ecosystems, therefore nitrogen is a limiting factor for plant growth in many cases. Nitrogen availability is usually related to the speed of key processes and nutrient fluxes between the ecosystem pools, therefore it is important to study the nitrogen biogeochemical cycle.

Nitrogen enters the internal forest cycle mainly through atmospheric nitrogen (N_2) fixation by bacteria and atmospheric wet and dry nitrogen deposition. In the other hand, plants assimilate atmospheric C in the form of carbon dioxide (CO_2) through photosynthesis, and emit a part of it because of cell respiration. The return of nutrients from the plants to the soil is driven by litterfall. It is the result of the plant material senescence minus the nutrient resorption, or retranslocation. The remaining biomass from the senescence process will be shed from the plant and become litter. Dead matter is broken down into simpler structures and molecules. Chemical transformations take place until the mineralization of nutrients. During the process, the volatile organic compounds (VOC) stored in the litter tissues are emitted to the atmosphere. During litter decomposition processes, carbon and nitrogen are mineralized and transformed into several molecule forms, but the decomposition also releases important quantities of CO_2, methane (CH_4), and nitrogen-based gases like nitrous oxide (N_2O). The soil nutrient pool serves as main nutrient source for plants, which are uptaken by the root system. Nutrients scape from the ecosystem through runoff and natural leaching.

The present thesis studies different processes relating plant litter and the biogeochemical cycles of carbon and nitrogen in several forest ecosystems

in Europe. This work unites different experiments in contrasting species (Scots pine, Douglas fir, cork oak) and climates (boreal, hemiboreal, atlantic and mediterranean).

Experiment sites

The studies were conducted in five sites along Europe. Hyytiälä (Finland), a Scots pine boreal forest; Järvselja (Estonia), a hemiboreal mixed forest; Männikjärve (Estonia), a peatland where drainage has been performed; Speulderbos (The Netherlands), an atlantic Douglas fir forest; Los Alcornocales (Spain), a mediterranean cork oak forest with contrasting atmospheric N deposition.

Main results

This work presents experiments investigating on processes directly related to plant litter production: leaf nitrogen resorption during senescence, dynamics of canopy fine litterfall, and the C and N inputs to forest floor. There were found differences in these traits according to site, climate, N deposition influence, and species characteristics.

High atmospheric N deposition at Los Alcornocales increased the green leaf N content. Higher leaf N concentration at this site could be related to the proximity of fossil-fuel power plants. Higher leaf N concentration was observed at Speulderbos, which is one of the European regions with highest load of N excess.

Nutrient resorption proficiency before litterfall (NRP: minimum annual leaf N concentration) was significantly smaller in Scots pine (Hyytiälä) than Douglas fir (Speulderbos) and cork oak (Los Alcornocales). This could suggest that the fertile soils at Speulderbos and Los Alcornocales, where there is high atmospheric N deposition, might compensate the incomplete resorption of these trees. Given results could explain observed high stand growth rate at Speulderbos (10.7 m^3 ha^{-1} yr^{-1}), despite high NRP values. Nutrient resorption efficiency values of Scots pine in Hyytiälä (64 %) and narrow fluctuation over the average annual senescent leaf N concentration, could suggest a more efficient use of the nitrogen in the N limited site of Hyytiälä.

The average (± SE) annual fine litter dry mass production for the study period was 2.86 ± 0.22 Mg ha^{-1} yr^{-1} in the Scots pine stand in Hyytiälä (1999-2011) and 4.34 ± 0.33 Mg ha^{-1} yr^{-1} in the Douglas fir stand in Speulderbos (2008-2010). Different annual litterfall dynamics were found between Scots pine, Douglas fir and cork oak. Annual average litter N concentration in Scots pine (Hyytiälä) was 0.601 ± 0.036 %, in Douglas fir (Speulderbos) was 1.7 ± 0.5 %, and in Järvselja 1.53 ± 0.10 %. Litter C concentrations were 50.27 ± 0.24 %, 53.1 ±1.1 %, and 48.65 ± 0.72 %, respectively. Litter N content fluctuated more along the year in Douglas fir than in Scots pine.

Nitrogen annual input through litterfall to Hyytiälä stand soil (15.9 ± 1.5 kg N ha^{-1} yr^{-1}) was much lower than in Speulderbos stand (75 ± 8 kg N ha^{-1} yr^{-1}), if compared with the C annual input (1430 ± 110 and 2300 ± 180 kg C ha^{-1} yr^{-1}, respectively). In Scots pine and Douglas fir, total litterfall, needle litterfall, total C and total N inputs to soil from litterfall were highly correlated.

Plant litter becomes part of the soil organic matter and minerals through decomposition. Plants and soil microbes release volatile compounds of C and N which leak out of the forest nutrient cycle. VOCs emitted by green leaves, litter layer, and soil were compared in a hemiboreal forest in Estonia. Monoterpene emission rate from soil was 40 times smaller (1.22 ± 0.16 nmol m^{-2} s^{-1}) than green leaf emission rate. As deciduous leaves mostly do not build specialized storage organs we could speculate that soil monoterpene emissions are caused by the coniferous litter. Seasonal differences in the total emission of VOCs were found, defined by a maximum in summer and a minimum in autumn and winter.

Peat bogs are areas poor in nutrients, where organic matter has been accumulated over the time. Therefore, they are a major store of terrestrial carbon. These ecosystems are sensitive to increase in soil temperature, because the stored carbon may be mobilized by bacteria and released to the atmosphere. Land needs to be drained if peat soils are used for silviculture, cropland, grassland and peat extraction. Peatland drainage mobilizes soil nutrients, speeds up the turnover of C and N, changes the soil properties, enhances tree growth, and produces volatile compounds. Lately, there is special attention to those volatile compounds because of their greenhouse effect (N_2O, CO_2 and CH_4) and therefore contribution

to global warming. This thesis presents a work on the soil greenhouse gas emission annual budget from a long-term (> 30 yr) drained peatland in comparison to the natural bog soil in Estonia.

At Männikjärve, long-term lowering of water table of the peatland, which successfully permitted afforestation and increased the stand density, made the ecosystem reach a new C and N cycle balance. The long-term drainage declined soil N_2O, CO_2 and CH_4 net emissions substantially. In terms of total climate forcing response, prolonged drought and long-term (> 30 yr) drainage consistently reduced the soils' contribution to greenhouse effect. However, substantial amounts of CO_2 were presumably lost from the drained peatland immediately after drainage. At Järvselja (another long-term drained area), the soil GHG emissions are nowadays similar to other boreal forests, supporting the idea of achievement of a new balance between the C and N ecosystem pools.

SUMMARY IN ESTONIAN

EUROOPA METSAÖKOSÜSTEEMIDE SÜSINIKU- JA LÄMMASTIKURINGE SEOSED TAIMSE VARISEGA

Süsinik (C) ja lämmastik (N) on biosfääri tähtsad makrotoitained, millel on ökosüsteemides väga erinevad funktsioonid. Biogeokeemia alastes uuringutes keskendutakse toitainete ringluse orgaanilise ja anorgaaniliste faasi vaheldumisele ja nende protsesside kiirusele. Hoolimata molekulaarse lämmastiku (N_2) küllusest atmosfääris, limiteerib taimede kasvu sageli lämmastikupuudus, sest alles ökosüsteemis fikseeritud lämmastik on taimedele toitainena kättesaadav. Tavaliselt seostatakse lämmastiku kättesaadavust ökosüsteemis toitainete liikumisega nende allikate vahel ning vastavate protsesside kiirusega. Seetõttu on lämmastikuringe biogeokeemiliste protsesside uurimine oluline.

Õhulämmastik (N_2) siseneb metsa aineringlusesse bakterite poolt fikseerituna ning märg- ja kuivdepositsiooni kaudu. Õhusüsinikku (C) assimileerivad taimed fotosünteesi käigus süsinikdioksiidist (CO_2) ning eraldavad osa sellest hingamise kaudu tagasi õhku. Toitainete tagastamine taimest mulda toimub tänu varisele, millest arvatakse maha toitainete vananevatest lehtedest tagasiimendumine. Vananenud lehed kukuvad taimedelt maha ning moodustavad varise. Surnud taimne materjal muutub kõdunemise käigus lihtsamateks struktuurideks ja molekulideks. Keemiline muundumine kestab toitainete mineraliseerumiseni. Lagunemisprotsessi käigus erituvad õhku taimekudedesse kogunenud lenduvad orgaanilised ühendid (VOC). Süsinik ja lämmastik mineraliseeruvad kõdu lagunemise käigus ning muundatakse erinevateks molekulideks. Selle protsessi käigus vabastatakse ka olulistes kogustes süsinikdioksiidi (CO_2), metaani (CH_4) ja lämmastikupõhiseid gaase nagu dilämmastikoksiid (N_2O). Taimed saavad juurte kaudu toitained mulla toitainete varust. Leostumine ja üleujutused viivad toitaineid mullast välja.

Antud uurimistöö käsitleb erinevaid taimekõdu ning süsiniku ja lämmastiku biogeokeemilise ringega seotud protsesse mitmetes Euroopa metsaökosüsteemides. Töö ühendab eksperimente, mis on läbi viidud kontrastselt erinevate puuliikidega nagu harilik mänd (*Pinus sylvestris*), harilik ebatsuuga (*Pseudotsuga menziesii*) ja korgitamm (*Quercus*

suber), mis on kasvanud erinevates kliimapiirkondades (boreaalne, hemiboreaalne, atlantiline, vahemereline).

Katsealad

Uuringuteks valiti viis paika Euroopas: Hyytiälä (Soome) harilik boreaalne männimets; Järvselja (Eesti) hemiboreaalne segamets; Männikjärve (Eesti) kuivendatud rabamaastik; Speulderbose (Holland) atlantiline hariliku ebatsuuga mets; Los Alcornocalese (Hispaania) vahemereline korgitamme mets, mille eri osades toimub atmosfäärse lämmastiku kogunemine väga erinevalt.

Peamised tulemused

Käesolevas töös esitletakse eksperimente, mis uurivad taimse varise tekkega seotud protsesse: taime poolt lehtede vananemisel toimuvat lämmastiku taasomastamist, võra peenvarise dünaamikat ja süsiniku ning lämmastiku lisandumist metsaalusesse pinnasesse. Sõltuvalt eksperimendi läbiviimise kohast, kliimast, lämmastiku saadavusest ja uuritavast taimeliigist leiti antud tunnustel erinevusi.

Atmosfääri suurem lämmastikusisaldus Los Alcornocalesis suurendas roheliste lehtede lämmastikusisaldust. Lehtede kõrgemat lämmastikusisaldust selles paigas võiks seostada fossiilkütuseid põletavate elektrijaamade lähedusega. Samuti tuvastati kõrgem lämmastiku kontsentratsioon Speulderbos, mis on üks suurema lämmastiku liiaga piirkondi Euroopas.

Toitainete taasomastamise ulatus enne lehtede langemist (NRP: vähim aastane lehtede lämmastiku kontsentratsioon) osutus oluliselt väiksemaks harilikul männil (Hyytiälä) võrreldes hariliku ebatsuuga (Speulderbos) ja korgitammega (Los Alcornocales). Tulemus lubab oletada, et Speulderbose ja Los Alcrnocalese viljakad mullad ning suur atmosfäärilise lämmastiku hulk võivad kompenseerida seal kasvavate puuliikide ebatäielikku N lehtedest tagasi imendumist ning seletada Speulderbose puistu suurt kasvukiirust (10.7 m^3 ha^{-1} a^{-1}) hoolimata kõrgetest NRP väärtustest. Toitainete lehtedest tagasiimendumise efektiivsus harilikul männil Hyytiäläs (64 %) ja väike aastaringne vanade lehtede lämmastiku kontsentratsiooni kõikumine viitab tõhusamale lämmastiku kasutusele Hyytiälä lämmastikuvaesetes tingimustes.

Töö läbiviimise ajal oli aasta lõikes mõõdetud keskmine (± SE) peenvarise kuivmassi produktsioon Hyytiälä hariliku männi kasvualal 2.86 ± 0.22 Mg ha^{-1} a^{-1}(1999-2011) ja Speulderbos'i katseala hariliku ebatsuuga kasvualal 4.34 ± 0.33 Mg ha^{-1} a^{-1} (2008-2010). Aastane varise dünaamika oli harilikul männil, harilikul ebatsuugal ja korgitammel erinev. Aasta keskmised varise lämmastikusisaldused olid järgmised: harilikul männil (Hyytiälä): 0.601 ± 0.036 %, harilikul ebatsuugal (Speulderbos): 1.7 ± 0.5 % ja Järvseljal: 1.53 ± 0.10 %. Varise süsinikukontsentratsioonid olid vastavalt: 50.27 ± 0.24 %, 53.1 ±1.1 %, ja 48.65 ± 0.7 %. Varise keskmine lämmastikusisaldus varieerus aasta lõikes harilikul ebatsuugal rohkem kui harilikul männil.

Varise lagunemisest tingitud aastane lämmastiku lisandumine metsaalusesse pinnasesse Hyytiälä katsealal oli tunduvalt madalam (15.9 ± 1.5 kg N ha^{-1} a^{-1}) kui Speulderbose katsealal (75 ± 8 kg N ha^{-1} a^{-1}) ning aasta keskmine süsiniku lisandumine mullale oli vastavalt 1430 ± 110 and 2300 ± 180 kg C ha^{-1} a^{-1}. Hariliku männi ja hariliku ebatsuuga puhul oli tugev korrelatsioon kogu varise ja okaste varise hulga ning kogu varise lagunemisest tuleneva mullale lisandunud süsiniku ja lämmastiku hulga vahel.

Taimne varis laguneb ja muutub mulla orgaaniliseks ning mineraalseks osaks. Taimede ja mulla mikroobid vabastavad lenduvate orgaaniliste ühenditena süsinikku ja lämmastikku ning see lahkub metsa toitaineringlusest. Rohelistest lehtedest, varisest ja pinnasest eralduvate orgaaniliste lenduvühendite võrdlus viidi läbi Eesti parasvöötme metsas. Monoterpeene eritus pinnasest 40 korda vähem (1.22 ± 0.16 mmol m^{-2} s^{-1}) kui rohelistest lehtedest. Kuna heitlehiste taimede lehtedel pole enamasti spetsiaalseid lenduvühendite säilitamiseks vajalikke struktuure, siis võiks järeldada, et pinnasest erituvad monoterpeenid pärinevad okaspuude varisest. Lenduvate orgaaniliste ühendite emissioonil leiti ka aastaajaline erinevus, mille maksimum on suvel ja miinimum sügisel ning talvel.

Turbarabad on toitainevaesed piirkonnad, kuhu aja jooksul on kogunenud orgaanilist ainet. Seetõttu on nad ühed suuremad maa süsinikuvaru säilitajad. Need ökosüsteemid on tundlikud pinnase temperatuuri tõusule, sest salvestatud süsinik võidakse bakterite poolt atmosfääri vabastada. Selleks, et turbamuldasid metsakasvatuseks, põllumaaks, rohumaaks ja turbatootmiseks kasutada on vaja pinnas kuivendada.

Turbamuldade kuivendamine vabastab toitaineid, kiirendab süsiniku ja lämmastiku ringlust, muudab mulla omadusi, ergutab puude kasvu ning toodab lenduvühendeid. Viimasel ajal on erilist tähelepanu pööratud nendele kasvuhooneefekti tekitavatele lenduvühenditele (NO_2, CO_2 ja CH_4), mis mõjutavad globaalset soojenemist. Antud doktoritöö käsitleb Eesti pikaajaliselt (>30. a.) kuivendatud turbarabade kasvuhoonegaaside aastaringset eritumist võrreldes looduslike rabadega.

Pikaajaline veetaseme alandamine Männikjärve rabas võimaldas taasmetsastamist, suurendas puistu tihedust (tk/ha) ning viis ökosüsteemi uuele süsiniku ja lämmastikuringe tasakaalule. Pikaajaline kuivendamine vähendas oluliselt pinnase NO_2, CO_2 ja CH_4 eritamist. Kliima mõjutamise seisukohast vähendab pikaajaline põud ja kuivendus järjekindlalt panust kasvuhooneefekti. Oluline kogus CO_2 lendus siiski kuivendusalalt eeldatavasti vahetult pärast kuivendamist. Järvseljal, mis on samuti pikaaegselt kuivendatud ala, on pinnase kasuhoonegaaside eraldumine praeguseks sarnane teiste boreaalsete metsadega, mis kinnitab uue süsiniku ja lämmastiku tasakaalu teket ökosüsteemis.

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Nutritional status of *Quercus suber* populations under contrasting tree dieback

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Summary

'Los Alcornocales' Natural Park (southwest Spain), one of the most important Mediterranean cork oak (*Quercus suber*) forests in Europe, has experienced a substantial tree dieback in the past decades. We hypothesize that areas experiencing high eutrophication should exhibit higher leaf nitrogen (N) concentrations and lower N resorption efficiency and proficiency indices than areas under lower environmental stress, and this could be related with forest functioning and tree mortality rates. To test this hypothesis, we collected leaf and soil samples from four plots in the southern part of the park, near a polluted industrial area, and four plots in the northern part of the park, far from this pollution source. N and phosphorus (P) in green and senescent leaves and soil NH_4^+-N, NO_3^--N and PO_4^{3-}-P were analysed. Foliar N and the leaf N:P ratio were significantly higher in the southern plots (high-mortality plots) than in the northern plots. The resorption efficiency was significantly higher for P than for N in all southern and northern plots. The values of resorption proficiency indicated incomplete resorption for N and complete resorption for P in all plots. Our results suggest that oak populations showing high-mortality rates may be associated with tree N and P imbalance.

Introduction

Los Alcornocales Natural Park, the most extensive Mediterranean cork oak forest in Europe, has experienced substantial tree mortality during the past three decades (Cano *et al.*, 2006). In the southern part of the park, the most affected area, cork oak density has decreased from the normal 400–600 trees ha^{-1} to fewer than 50 trees ha^{-1} (Figure 1). Despite the increasingly visible damage in oak woodlands, the role of the various environmental factors in their decline is not clear (Costa *et al.*, 2010). The oak dieback has been related to an increase in susceptibility to pathogen attacks as a consequence of intensive forest management and global change (Brasier, 1996). Anthropogenic nitrogen (N) deposition in N-limited ecosystems is a primary component of global change. During the twentieth century, anthropogenic N fixation doubled the global flux of N to the biosphere. Moreover, N deposition rates are expected to increase another two or threefold before reaching a plateau (Vitousek *et al.*, 1997; Galloway and Cowling, 2002; Lamarque *et al.*, 2005). If the critical load is exceeded, possible scenarios include eutrophication of soils and waters (Emmett and Reynolds, 1996). Critical loads are defined as the amount of deposition of a substance that can be tolerated by an ecosystem without incurring harm and are used in Europe and Canada as an assessment tool for determining emissions targets for sulphur (S) and N pollution (Burns *et al.*, 2008)

In the last 20 years, the European Monitoring and Evaluation Programme network has detected substantial N and S emission and deposition rates near southern areas of the Alcornocales Natural Park (Figure 2, Fagerli, 2005), where the highest tree mortality rate has been reported. An unknown percentage of emitted pollutants are locally generated in a nearby industrial area, which includes a carbon-based power plant. N deposition has important effects on ecosystem function, and it has been related to forest dieback and disease emergence in humans and wildlife worldwide (Johnson *et al.*, 1999; Bobbink *et al.*, 2010). Anthropogenic eutrophication has also changed the relative importance of nutrient elements in limiting productivity (Verhoeven *et al.*, 1996; Aerts and Bobbink, 1999; Beltman *et al.*, 2000; Fourqurean and Zieman, 2002). Aber *et al.* (1989, 1998) proposed a model describing the

Figure 1. Satellite images of the study area. Northern plots (top image) and southern plots (bottom image).

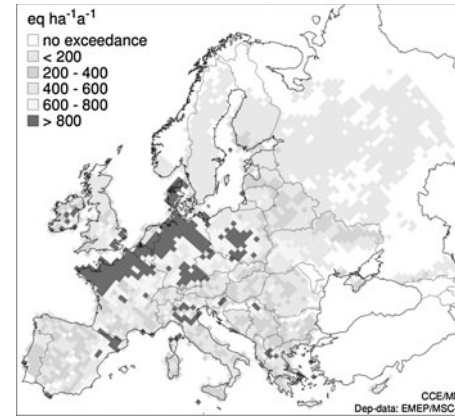

Figure 2. Load of excess nutrients in Europe in 2003 (Fagerli, 2005).

long-term consequences of elevated N deposition on forest ecosystems and the progressive development of N saturation. Based on this model, N availability and leaf N concentration should increase with increasing atmospheric N deposition. As a consequence, plant physiological traits, such as leaf nutrient resorption efficiencies, may also be affected. Nutrient resorption can also be quantified by resorption proficiency, a parameter describing the level to which a nutrient is reduced during senescence (Killingbeck, 1996), with higher proficiencies corresponding to lower final nutrient concentrations. These conservation mechanisms are particularly important for N and phosphorus (P) for which half or more of the maximum leaf content is typically resorbed to other parts of the tree before leaf abscission (Aerts, 1996; Côté *et al.*, 2002). Although there is abundant information on the response of resorption indices to changes in soil fertility, there is no information on the changes in these leaf traits in areas experiencing forest dieback, despite the direct relationship between forest dieback and the increase of atmospheric N deposition and acid rain. We know that N additions to soil can directly or indirectly cause nutrient imbalances and even tree mortality (Schulze 1989; McNulty *et al.*, 1996). However, whether physiological traits such as nutrient resorption efficiency or proficiency are responsive to this type of disturbance has not been described before.

In evergreen plants, leaf resorption is considered to be biochemically complete if the nutrient concentration in senescent leaves falls below 0.70 and 0.04 per cent for N and P, respectively (Killingbeck, 1996). Levels above 1.0 and 0.05 per cent of these nutrients indicate incomplete resorption and reveal a low capacity for nutrient resorption. The capacity for nutrient resorption is defined as nutrient proficiency. Nutrient proficiency may reflect changes in nutrient availability to a greater extent than it reflects nutrient resorption efficiency. These different measures of resorption are often complementary (Killingbeck, 1996; Killingbeck and Whitford, 2001).

Our goals were to compare the leaf nutrient content and nutrient resorption efficiency and proficiency indices in two *Quercus suber* populations under contrasting environmental stress. We hypothesize that *Q. suber* trees located close to the sources of N and S emissions should exhibit higher leaf N concentrations and lower N resorption efficiency and proficiency indices than plots under lower environmental stress and this could be related with forest

functioning and tree dieback. However, we do not expect differences in leaf P concentrations and P resorption indices between the two oak populations since soil acidity more than N availability should drive P availability in these soils.

Materials and methods

Study area

The study area is located in the Alcornocales Natural Park in southwest Spain (36° 21′ N; 5° 32′ W). A large industrial area (including coal-burning power plants) located near the southern part of the park has generated significant long-term N and S emissions and an enhanced local atmospheric deposition of these nutrients. These conditions have produced one of the highest estimated N and S excesses in Europe (Figure 2, Fagerli, 2005). A high rate of cork oak mortality was observed during recent years in the southern part of the park. In some areas, over 80 per cent of the tree cover has disappeared during the past 10 years (Figure 1). In the northern part of the park, scattered tree mortality (<5 per cent of the population of trees) was also observed (Tuset and Sanchez, 2004).

The Alcornocales Natural Park is under the influence of a Mediterranean climate, but the park is also affected by its proximity to both the Mediterranean Sea and the Atlantic Ocean. These bodies of water buffer the variations in temperature produced by the Mediterranean climate. The proximity of the park to both water bodies also increases the mean annual precipitation. These mean values range between 800 and 1400 mm at the higher altitudes (600–800 m). The park's sandy soils are classified as Eutric cambisols (FAO, 1994). Soils show a typical A(B)C profile. The physical and chemical characteristics of these soils are shown in Table 1. The tree stratum is dominated by *Q. suber* L. (cork oak). *Q. canariensis* Willd. and *Q. pyrenaica* Willd. are occasionally present. The understory layer is composed mainly of *Allium triquetrum* L., *Lavandula stoechas* L., *Calycotome villosa* Poiret. and *Phlomis purpurea* L.

Table 1: Soil physical and chemical properties and characteristics data for leaf contents in *Quercus suber* as determined at two sites in Los Alcornocales Natural Park

	South plots	North plots
Soil		
Clay (%)	3.76 ± 1.25	5.59 ± 3.1
Silt (%)	8.79 ± 3.77	19.88 ± 2.49
Sand (%)	87.45 ± 2.52	74.53 ± 0.61
Water content (%)*	14.63 ± 1.74	15.04 ± 1.78
pH	5.05 ± 0.40	5.81 ± 0.31
Ca (meq $100g^{-1}$)	1.08 ± 0.27	2.35 ± 0.72
Mg (meq $100g^{-1}$)	0.53 ± 0.04	1.07 ± 0.01
K (meq $100g^{-1}$)	0.16 ± 0.07	0.35 ± 0.004
Na (meq $100g^{-1}$)	0.26 ± 0.13	0.38 ± 0.04
Organic matter (%)	2.33 ± 0.07	2.32 ± 0.23
Acetic acid PO_4^{3-}-P ($\mu g\ g^{-1}$)	1.43 ± 0.14	2.25 ± 0.32
NH_4^+-N ($\mu g\ g^{-1}$)	4.93 ± 0.58	3.93 ± 0.43
NO_3^--N ($\mu g\ g^{-1}$)	2.05 ± 0.41	3.58 ± 0.91
Total N (%)	0.169 ± 0.008	0.1643 ± 0.0075
C/N	7.99 ± 0.14	8.29 ± 0.46
Foliar		
Leaf mass (mg)	0.153 ± 0.005	0.156 ± 0.004
Leaf area (mm^2)	1015 ± 33	1077 ± 33
LMA ($g\ cm^{-2}$)	15.04 ± 0.31	14.59 ± 0.23

Values are presented as mean ± SE.

* Water content is the mean of this parameter measured from collected soil samples.

Sampling design

Two contrasting sites (the North and the South plots) were selected in the park. The sites were chosen based on their distance from the main source of air pollution (an industrial area including power stations) and their observed rates of forest-level dieback. The North plots are located over 35 km from the N emission sources at an altitude of ca. 180 m, whereas the South plots are located 15 km from the emission sources at an altitude of ca. 80 m. Eight ca. 1-ha plots were established at each site – four with a southern aspect and four with a northern aspect to account for aspect-associated microclimatic conditions. In each plot, five cork oak trees were randomly selected for sampling, and five sets of samples were collected during 2007. The sampling dates were 23–24 February, 13 April, 25 May, 10 July and 2 November. The same trees were sampled each time. Measurements of photosynthetic efficiency were taken around the canopy, including sun and shade leaves, with a Hansatech fluorometer (Baker, 1991). Trees showing a PSII efficiency (Fv/Fmax) below 0.8 were considered to be ill and were therefore omitted from the sampling. Ill trees were excluded because tree disease may modify the nutritional status of trees. Around each selected individual (less than 2 m from the tree trunk), three soil samples were taken from the top 10 cm of the soil profile and combined to form one composite sample per tree. Simultaneously, the soil water content was measured by using a time-domain reflectometry technique. At each tree and sampling date, green leaves were sampled from the lower part of the canopy, including sun and shade leaves and collecting one composite sample for each tree. On the May sampling date (coinciding with the leaf-fall peak), additional senescent leaves were collected by gently shaking the tree branches. Soil and leaf samples were transported in polyethylene bags in coolers to the laboratory. Samples were stored at 3°C in laboratory refrigerators and processed as soon as possible (less than a week in all cases).

Laboratory analysis

The oak leaves were oven-dried (80°C), weighed and scanned to calculate the leaf surface area by using UTHSCSA Image Tool 3.0 software (http://ddsdx.uthscsa.edu/dig/pub/IT/IT3.zip). Later, the leaf samples were milled to pass through a 2-mm mesh screen and stored for subsequent N and P chemical analysis. The leaf samples were digested

with a sulfuric peroxide mix following Allen *et al.* (1986). The N and P concentration in the digested aliquots were estimated by colourimetric methods (the indophenol blue method and the molybdenum blue method, respectively) by using a microplate reader (Sims *et al.*, 1995).

The soil samples were oven-dried (80°C), milled and sieved to pass through a 2-mm mesh screen. The soil texture was estimated with the hydrometer method, following Kroetsch and Wang (2008). The pH and soil conductivity were measured in 1:2.5 and 1:5 soil–water solutions, respectively. The soil organic matter was analysed by wet oxidation techniques, following Skjemstad and Baldock (2006).

The carbonate levels were analysed according to Boon Goh and Mermut (2007). The total soil N was measured by standard Kjeldahl procedures (Rutherford *et al.*, 2007). To measure soil N, soil subsamples (20 g) were extracted with 100 ml of 0.5 M K_2SO_4 and shaken for 1 h. The extract was filtered with a 0.45-μm Millipore filter. The NH_4^+-N and NO_3^--N levels in the extracts were measured by colorimetric method (indophenol blue) using a microplate reader (Sims *et al.*, 1995). The soil PO_4^{3-}-P was extracted with 100 ml of 2.5 per cent CH_3CO_2H, and the concentration in the extract was determined by the molybdenum blue colorimetric method (Allen *et al.*, 1986). For acid soils, this extraction is equivalent to the standard 0.5 M sodium bicarbonate method used in neutral and basic soils (Hedley *et al.*, 1982; Allen *et al.*, 1986).

Numerical analysis

The N and P resorption efficiencies were calculated as the difference between the annual maximum leaf nutrient concentration in green leaves and the leaf nutrient concentration in senescent leaves and expressed as percentage of the green leaf concentration per plot. The N and P resorption efficiencies were calculated per unit of leaf surface to minimize the effect of mass loss during senescence (Poorter *et al.*, 2009). The nutrient resorption proficiency was defined as the minimum N or P concentration per unit of leaf mass found in senescent leaves (Killingbeck, 1996). Values of N and P resorption proficiency above 1 and 0.05 per cent, respectively, were considered to represent incomplete resorption. Complete resorption was defined as a resorption proficiency value below 0.7 per cent for N and 0.04 per cent for P (Killingbeck, 1996).

The differences in soil and leaf variables among plot locations and sampling dates were analysed by linear mixed-effects models. Normality and homogeneity of variance tests (Shapiro–Wilk and Levene tests) were performed prior to analysis, and the variables were log transformed as necessary. Plot location was treated as a fixed effect and the sampling dates were treated as random effects. Comparisons between the nutrient resorption indices found in different plots were made with Kruskal–Wallis rank sum test. We used a significance level of $P < 0.05$.

Results

The foliar N content was significantly higher in the South plots (high-mortality plots) than in the North plots ($F = 6.15$, df = 31, $P = 0.019$, Figure 3). Nevertheless, the foliar

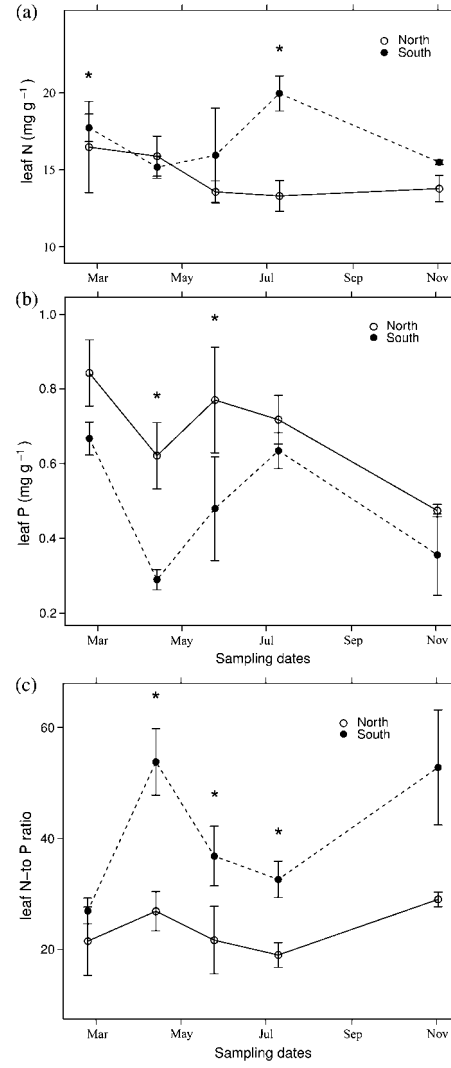

Figure 3. Leaf N (a), leaf P (b) and leaf N:P ratio (c) in the South and the North cork oak plots in Los Alcornocales Natural Park.

P content was significantly lower in the South plots than in the North plots ($F = 16.57$, df = 31, $P = 0.0002$). The N:P ratio was significantly higher in the South plots than in the North plots ($F = 35.25$, df = 31, $P < 0.0001$). No significant differences were found in leaf mass ($F = 0.36$, df = 29, $P = 0.550$), leaf area ($F = 2.24$, df = 29, $P = 0.145$) or leaf mass per area (LMA, $F = 0.49$, df = 29, $P = 0.486$) between the South and the North plots. Sampling dates had no effect on any of the leaf variables; however, slope aspect showed significant effect on the leaf P and the leaf N-to-P ratio (Table 2).

Table 2: Linear mixed-effects model of leaf traits as dependent variable and plot and sampling date and aspect as independent variables

	Num DF	Den DF	*F*-value	*P*-value
Leaf N				
Intercept	1	31	8836.7131	0
Plot	1	31	6.1469	0.0188
Date	1	3	1.2128	0.3512
Plot:aspect	1	31	1.9659	0.1708
Plot:date	1	31	1.3300	0.2576
Plot:aspect:date	1	31	0.0412	0.8403
Leaf P				
Intercept	1	31	22.7487	4.15×10^8
Plot	1	31	16.5719	0.0002
Date	1	3	0.7842	0.4410
Plot:aspect	1	31	5.2582	0.0287
Plot:date	1	31	0.1590	0.6927
Plot:aspect:date	1	31	0.0952	0.7596
Leaf N:P				
Intercept	1	31	895.4036	0
Plot	1	31	35.2543	1.47×10^8
Date	1	3	0.5343	0.5177
Plot:aspect	1	31	11.6130	0.0018
Plot:date	1	31	0.1575	0.6941
Plot:aspect:date	1	31	0.0457	0.8319
Leaf mass				
Intercept	1	29	1223.2565	0
Plot	1	29	0.3650	0.5504
Date	1	3	2.3558	0.2223
Plot:aspect	2	29	0.4999	0.6117
Plot:date	1	29	0.1035	0.7499
Plot:aspect:date	2	29	1.0406	0.3660
Leaf area				
Intercept	1	29	640.6563	0
Plot	1	29	2.2389	0.1453
Date	1	3	1.3261	0.3329
Plot:aspect	2	29	0.1176	0.8894
Plot:date	1	29	1.1802	0.2862
Plot:aspect:date	2	29	1.0781	0.3534
LMA				
Intercept	1	29	645.1984	0
Plot	1	29	0.4982	0.4858
Date	1	3	0.0001	0.9921
Plot:aspect	2	29	0.2395	0.7885
Plot:date	1	29	0.1330	0.7179
Plot:aspect:date	2	29	0.0552	0.9463

Aspect is a nested variable within plots. Date was included as repeated measurements within subjects.

The P resorption efficiency was significantly higher than the N resorption efficiency at both sites (Figure 4, Chi-square = 11.29, df = 1, $P = 0.0008$). The mean leaf N resorption efficiency was 34 per cent for the North plots and 42 per cent for the South plots (Figure 4a). The mean leaf P resorption efficiency was 87 and 83 per cent for the North and the South plots, respectively (Figure 4b). No significant differences were observed for either N or P resorption efficiency between the North and the South plots (Chi-square = 2.1, $P = 0.1489$, chi-square = 0.75, $P = 0.3865$).

The N resorption proficiency values indicated incomplete resorption for most oak individuals in both the North and the South plots (Figure 4c). However, the P resorption proficiency values showed that P resorption was complete for all oak individuals (Figure 4d). No significant differences between the North and the South oak populations were found for N ($F = 1.5$, df = 1, $P = 0.266$) or P resorption proficiency ($F = 0.41$, df = 1, $P = 0.547$).

The mean soil NH_4^+-N, NO_3^--N and PO_4^{3-}-P values were very low in both the South and the North plots (Table 1), but significantly higher values were found in the North plots for soil N-NO_3^-. No other significant differences in soil properties were found between the South and North plots.

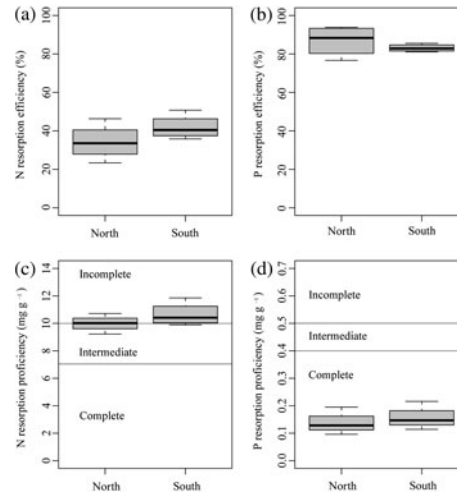

Figure 4. (a) N resorption efficiency (calculated per unit of leaf surface) in the South and the North cork oak plots. (b) P resorption efficiency (calculated per unit of leaf surface) in the South and the North cork oak plots. (c) N resorption proficiency in the South and the North cork oak plots. (d) P resorption proficiency in the South and the North cork oak plots. The figure shows the median and the 10th, 25th, 75th and 90th percentiles. The horizontal lines show the threshold values of resorption proficiency, following Killingbeck (1996). Outliers (atypical points) were defined using 1.5 times the difference between the third and first quartiles.

Discussion

As we predicted, *Q. suber* trees exhibited higher leaf N concentrations and higher leaf N:P ratios in the South (high-mortality) plots than in the North plots. However, we did not find significant differences in the resorption efficiency or proficiency indices between the North and South plots. The higher leaf N concentration in the South plots may be related to the proximity of fossil-fuelled power plants, which have been responsible for the high N deposition rates detected over the past 30 years. An increase in foliar N concentrations and nitrate assimilation is one of the symptoms of N saturation (Aber and Melillo, 2001; Tessier and Raynal, 2003; Lovett and Goodale, 2011).

Interestingly, in the South plots, the leaf P was significantly lower than in the North plots, suggesting lower P availability in the South plots. Interactions between N and P are particularly important in determining the nature of nutrient limitation in the vast majority of systems (Townsend *et al.*, 2010). Human activity can induce P limitation by affecting the supply of N (Vitousek *et al.*, 2010). For example, extraordinarily high levels of atmospheric N deposition in northwestern Europe have removed N limitation at many sites (Berendse *et al.*, 1993; Stevens *et al.*, 2004; Vitousek *et al.*, 2010), and many of the altered systems are now limited primarily by P (Verhoeven and Schmitz, 1991; Aerts *et al.*, 1992). In areas receiving high levels of anthropogenic N deposition, the high N level allows organisms to produce more extracellular phosphatase enzyme and thereby release phosphate from soil organic matter (Olander and Vitousek, 2000; Treseder and Vitousek, 2001). However, the increase in P cycling observed at sites with high N deposition typically is insufficient to balance the increased rate of N input, and P often becomes limiting (Mohren *et al.*, 1986; Teng and Timmer, 1995; Fenn *et al.*, 2006). Therefore, a secondary effect of increased N availability in N-limited ecosystems may be a shift towards P limitation (Bobbink *et al.*, 1998). This shift may be seen in lower P concentrations in litter owing to growth dilution of the P in the leaves and increased P resorption (Shaver and Melillo, 1984; Vitousek, 1984; Kemp *et al.*, 1994). Interestingly, we found a significant effect of aspect within plots on leaf P, with the highest P levels associated with the southern aspects, which may suggest a connection between P and C availability at the tree level.

N:P ratios have been used as diagnostic indicators of N saturation (Fenn *et al.*, 1996) and to identify the thresholds of nutrient limitation (Penning de Vries *et al.*, 1980; Koerselman and Meuleman, 1996; Verhoeven *et al.*, 1996; Aerts and Chapin, 2000; Güsewell and Koerselman, 2002). Based on studies of European wetland plants, the thresholds of foliar N:P ratios were found to be <14 for N limitation and >16 for P limitation. Tessier and Raynal (2003) demonstrate that these N:P ratios can be equally effective predictors of nutrient limitation in upland ecosystems. Therefore, N:P ratios can be used for management and monitoring purposes in evaluating the nutrient status of upland ecosystems, with particular relevance to the continued deposition of elevated atmospheric N and to the diagnosis of N saturation. Thus, N:P ratios have been used to detect N saturation in ecosystems in the western US (Fenn *et al.*, 1996,1998; Williams *et al.*, 1996).

The N:P ratio in this study indicated that P limitation was present in both the North and the South plots. However, while in the North plots, these ratios reached values between 20 and 30 and in the South plots, these values ranged between 30 and 50, suggesting a stronger P limitation in the South plots. This finding also suggested that these trees may be closer to the N saturation stage, a condition that would correspond to the high rates of tree mortality found in this area.

The resorption of nutrients from senescent leaves is of great adaptive significance because such nutrients are directly available for further use and therefore make a species less dependent on current nutrient uptake (Aerts and Chapin, 2000). It has often been suggested that species from low-nutrient habitats have higher nutrient resorption efficiencies (van Heerwaarden *et al.*, 2003). However, high nutrient resorption efficiency appears not to be very responsive to changes in nutrient supply (Aerts, 1996; Aerts and Chapin, 2000). Resorption proficiency seems to be more responsive than resorption efficiency to nutrient availability. Several studies reported that N fertilization leads to lower N resorption proficiencies (Shaver and Melillo, 1984; Kemp *et al.*, 1994; Vitousek *et al.*, 1998). This relationship between N resorption proficiency and N availability is also found along natural fertility gradients (Pugnaire and Chapin, 1993; Eckstein *et al.*, 1999). However, we did not find significant differences in nutrient resorption efficiencies or resorption proficiencies between the North and the South plots. Thus, these physiological traits do not seem to be affected by the different nutritional status of the trees and do not seem to be related to different tree mortality rates.

The N resorption efficiencies at both sites had low values compared with the average N resorption efficiency (50 per cent) found by a large literature survey (Aerts, 1996). However, the P resorption efficiencies were above 80 per cent in both the North and the South plots. Moreover, the resorption proficiencies were classified as incomplete for N and complete for P in both the North and the South plots. These results again suggest P limitation in the study area.

Despite the differences in leaf N and P between the North and the South plots, we could not detect any significant differences in soil NH_4^+-N and PO_4^{3-}-P between the North and the South plots. The only differences were found in soil N-NO_3^-, but higher levels were found in the North plots. The soil N and P extractable pools show high spatial and temporal variability in most ecosystems (Rodríguez *et al.*, 2009), and therefore, a single sampling date in each season probably does not yield enough statistical power to detect differences between plots. Furthermore, extractable soil nutrient pools are a poor estimate of nutrient availability, and the measurement of mineralization or nitrification rates may be more adequate and conclusive for demonstrating differences between sites. We do not have a clear explanation for the higher NO_3^--N levels found in the North plots. However, the lower tree

density in the south plots might have favoured the leaching and denitrification of NO_3^--N from soil. These losses of N occur in response to ecosystem disturbances that disrupt the biological mechanisms of NO_3^--N retention (Vitousek and Howarth, 1991).

Our experimental design compared plots that showed high-mortality rates and were near high N emission sources with low-mortality plots far from these sources. Although all the plots were established on the same soil type and we did not find differences in leaf traits (e.g. leaf mass, leaf area or LMA and most of the soil variables), the different geographical locations and altitudes of the South and the North plots may have produced differences in microclimate that could have affected the tree phenology and the leaf nutrient concentration. Genetic differences between the North and the South cork oak populations are also unlikely because both populations form a single continuous distribution along the north–south axis of the park. Differences in tree density between plots may influence the nutritional status of the populations, but we cannot estimate this effect.

The differences in the leaf nutrient concentration found by this study are too large to be explained by minor geographical variations. More likely, they are related to the different stress conditions (both tree mortality and/or N deposition influence) affecting the study populations. Leaf nutrient concentrations are better indicators than soil nutrient variables because they show nutritional conditions across one or more growing seasons (Vitousek and Farrington, 1997; Aerts and Chapin, 2000; Hobbie and Gough, 2002; Vitousek, 2004; Townsend *et al.*, 2007).

We also expected that differences in leaf nutrient concentrations would be reflected in physiological processes, such as the resorption indices. However, these variables seem to be independent of the geographical location. This finding agrees with the weak association that other authors have found between resorption indices and changes in nutrient availability (Aerts and Chapin, 2000).

Conclusions

Our results suggest that although populations showing high-mortality rates may be associated with tree N and P imbalance, the resorption indices do not appear to be a good indicator of the nutritional status of trees showing different levels of forest dieback. However, more research is needed to establish a link between forest dieback and tree nutritional status.

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Conflict of interest statement

None declared.

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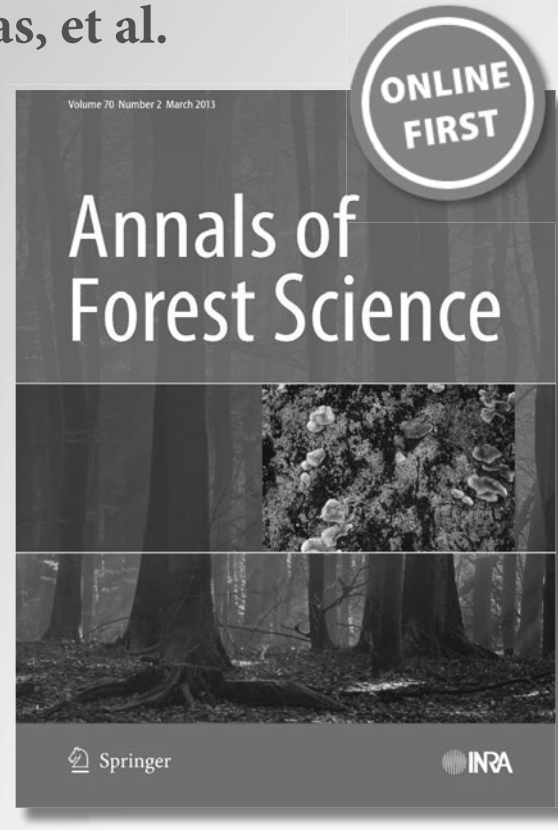

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ORIGINAL PAPER

Inter- and intra-annual variations in canopy fine litterfall and carbon and nitrogen inputs to the forest floor in two European coniferous forests

Miguel Portillo-Estrada · Janne F. J. Korhonen · Mari Pihlatie · Jukka Pumpanen · Arnoud K. F. Frumau · Lourdes Morillas · Tiina Tosens · Ülo Niinemets

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Abstract
• ***Context*** The amount and chemistry of litterfall have been known to strongly vary among the years with important implications for ecosystem nutrient cycles, but there are few quantitative data describing such variations.
• ***Aims*** We studied the climatic implications on the variation in litterfall and its C and N input to soil in two distinct European coniferous forests.
• ***Methods*** Year-to-year variations in canopy litterfall were measured in a Scots pine stand (Hyytiälä, Finland) over 13 years, and a Douglas fir stand (Speulderbos, The Netherlands) over 3 years.
• ***Results*** Important inter-annual variations in litterfall were observed in Scots pine. Litterfall was mainly driven by leaf senescence; however, premature needle fall was observed in high wind speed and early frost events. The seasonal variation in litterfall was characterized by a maximum in September in Scots pine, and by a biphasic variation pattern in Douglas fir, in May and November. Lower seasonal variations and lower annual average in litterfall N content were observed in Scots pine.
• ***Conclusion*** Significant inter- and intra-annual variations in litterfall and chemistry and between the sites were demonstrated; and it depended on year-to-year differences in climate and extreme weather events.

Keywords Litter production rate · Scots pine · Douglas fir · Temporal variability · Wind-influenced litterfall

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Contribution of the co-authors Miguel Portillo-Estrada: Original idea, design of the experiment, data analysis, writing the manuscript
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Mari Pihlatie: Running the experiment, supervising the manuscript
Jukka Pumpanen: Running the experiment, supervising the manuscript
Arnoud K. F. Frumau: Running the experiment, supervising the manuscript
Lourdes Morillas: Data analysis, supervising the manuscript
Tiina Tosens: Design of the experiment, supervising the manuscript
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1 Introduction

Litterfall is a key process in forest nutrient cycling, and it is the main above-ground contributor of carbon and nutrients to the forest floor (Vitousek et al. 1995). Total litter production and litter quality determine the soil microbial activity, and accordingly, the release of nutrients into the soil (Ganjegunte et al. 2004). Once the dead biomass is available for decomposition processes, leaching of nutrients by rainfall and biomass consumption by detritivores convert the biomass into a form that can be chemically used by bacteria and fungi (Aerts 1997). Thus, understanding the determinants of litter production and quality is of paramount significance for the understanding of nutrient turnover in forest soils (Melillo et al. 1989). Intra- and inter-annual variations in litter production and quality are also

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importantly related to tree nutrient status. Alterations of such dynamic phenomena are pertinent to study and need consideration in modeling long-term nutrient cycles.

In mature coniferous temperate and boreal forests, aboveground litter is produced by the trees (needles, twigs and branches, cones and dead stems), and by the understory vegetation. Unlike from winter-deciduous forests, where the major litter production occurs during autumn leaf fall, litter is continuously produced in evergreen conifer stands, although more or less distinct peaks of litter production can be often identified during the year. As an example, it is well-known that the oldest needle class of Scots pine sheds needles during August–October in boreal forests (Ukonmaanaho et al. 2008), whereas Douglas fir presents two peaks of needle litterfall in Spring and Autumn.

The canopy tree litter production varies over the years and its components have different dynamics and turnover times. For example: (1) tree stems can live for decades to centuries, their litter production is measurable (Aerts and Berendse 1989), but reliable estimations can be done only after long-time studies. (2) Branches may last from years to decades, their litter production and input to the soil system are relatively unknown (Lehtonen et al. 2004). (3) Needles are renewed every few years in evergreen conifers, thus this litter fraction can be best assessed; however, the variation of annual needle litterfall can be very large (up to 40 % in a mature Scots pine forest (Kouki and Hokkanen 1992)). These premises suggest that short-term studies showing 1-year data of litter trap studies seem to be far from the actual average budget of biomass cycling, and that all types of litter must be taken into account in studies of the C and N cycle in forest ecosystems. But for short-term studies, litter trap method gives an overview of the canopy litter production, the speed of nutrient cycling, and the nutrient accumulation in the reservoirs.

Year-to-year variations in climate and extreme weather events such as storms can importantly alter the dynamics and total amount of litter production (Lodge et al. 1991). While all above-ground biomass ultimately ends up as litter, such environmental effects and extreme events can importantly alter the timing of peak litter events (Álvarez et al. 2009), thereby altering the soil microbial activity and the nutrient cycle (Sanford et al. 1991). Furthermore, these factors can even more strongly alter litter quality (Pérez-Suárez et al. 2009). As nutrient resorption is a time-consuming event, premature foliage and twig loss as the result of storm events or early frosts can result in litter with higher nutrient concentration (Killingbeck 1996). Thus, years with premature litter fall can importantly alter soil activity and affect plant growth in the subsequent season. There are evidences of enhanced soil nutrient availability after storm-induced litterfall (Pérez-Suárez et al. 2009; Sanford et al. 1991).

In this study, we investigated year-to-year variations in the canopy litter production, which is highly important for long-term prediction of ecosystem nutrient dynamics. The study was conducted in two coniferous forests with contrasting average foliage longevity and climatic conditions to gain insight into the general and specific responses of litter production in two key conifer species. Scots pine, the dominant species in the northern site in Hyytiälä, Finland, has lower foliage retention time, on average 2 years (Wang et al. 2012), and supports lower leaf area index than Douglas fir, the dominant species in the Dutch site in Speulderbos that has an average foliar retention time of 2.6 years (Wang et al. 2012). We hypothesized that year-to-year variations in weather conditions importantly alter total litter production and litter quality. These effects could be more pronounced in the Douglas fir stand that supports larger number of foliage cohorts and overall more foliage area.

This study is based on a unique dataset of detailed leaf litterfall dynamics in two important conifer species in two European sites (13 years data in one and 3.5 years data in the other site). The results of such detailed long-term litterfall data demonstrate large year-to-year variations in litterfall dynamics and nutrient content that has potentially major significance for ecosystem performance.

2 Material and methods

2.1 Study sites

The study was conducted in two key sites of Nitroeurope IP project (for detailed description of the project and sites see Owen et al. 2011). See Table 1 for more site description.

The Hyytiälä Forestry Field Station (Kolari et al. 2009) is situated in Southern Finland, 60 km northeast from Tampere. It supports a homogeneous Scots pine (*Pinus sylvestris* L.) stand. This species is dominant in more than half of the forest area in southern Finland. Stand management has been conducted according to the standard silvicultural guidelines for similar forest types in Finland, and by now, the stand is half-way through the rotation time. The site has a relatively flat topography.

The Speulderbos site (Su et al. 2009), operated by the National Institute for Public Health and the Environment (RIVM), is located in the central Netherlands. It is approximately 25 km northeast from the city of Amersfoort, within a large forested area in the Netherlands. The site supports a dense 2.5-ha Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) stand. The site topography is slightly undulating with height variations of 10 to 20 m within distances of 1 km.

2.2 Litter collection

The study was conducted for a period of 156 months (January 1999–December 2011) in Hyytiälä, and 43 months (June 2007–December 2010) in Speulderbos. Litter traps (mesh size$<$0.5 mm) were placed above the topsoil. Twenty circular litter traps of 0.2 m^2 in Hyytiälä

Table 1 Site description

Site description	Hyytiälä[a]	Speulderbos[b]
Coordinates	(61°50′51″, 24°17′41″) elevation 180 m.a.s.l.	(52°15′08″, 05°41′26″) elevation 52 m.a.s.l.
Tree species	Scots pine	Douglas fir
Year of plantation	1962	1962
Soil type and texture	Haplic podzol	Orthic podsol
Soil texture	Till material dominated by fine sand	Silty sand texture
Soil depth	From 0 to 200 cm	309 cm
Soil pH	3.3	3.7
Stand density	1,400 ha^{-1} (2006)	785 ha^{-1} (2006)
Stand height	16.3 m (2006)	32 m (2006)
Stand growth rate	8 $m^3 ha^{-1} year^{-1}$	10.7 $m^3 ha^{-1} year^{-1}$
Leaf area index	6.5 $m^2 m^{-2}$ (all sided)	7.8–10.5 $m^2 m^{-2}$ (projected) [c]
Annual mean air temperature	3.5 °C	9.6 °C
Annual mean soil temperature	4.6 °C	9.4 °C
Precipitation	713 $mm year^{-1}$ (1971–2000) [d]	889 $mm year^{-1}$ (1958–2002) [e]
Annual maximum snow cover	50 cm (1997–2012)	15 cm (1994–2012)

[a] Kolari et al. (2009)
[b] Su et al. (2009)
[c] Van Wijk et al. (2000)
[d] Drebs et al. (2002)
[e] Reyer et al. (2010)

(Ilvesniemi et al. 2009) and 10 squared litter traps of 0.25 m^2 in Speulderbos were used. Distance between traps was 10 m. Tree litter was collected every ca. 30 days (every 3 months during 1999–2005 in Hyytiälä). Litter from each trap was enclosed in labeled plastic bags and transported to the lab. Samples were first air-dried at room temperature until constant mass. In Hyytiälä, during the monthly sampling in winter time, snow was carefully removed from the collectors so that snow did not flow over from the collectors. During winter, samples with snow cover were melted indoors. Litter was sorted manually with tweezers into four groups: needles, twigs (diameter<1 cm), bark, and cones; the sum of these groups is what we considered *canopy fine litter*. When litter fragments were difficult to identify due to their small size, they were considered the *rest* fraction. The rest fraction mainly consists of brittled twig bark and small needle pieces, including sometimes resin droplets. Twigs which diameter was >1 cm where not considered fine canopy litter, thus discarded in the total canopy litter values. After sorting, the mass of different litter fractions was determined after oven-drying at 60 °C to constant mass. Only the litter from the dominant conifer species was considered.

2.3 Chemical analysis

The samples from different litter traps were pooled to one sample per collection day before the chemical analysis. Total nitrogen and carbon contents of oven-dried samples were determined by dry combustion method separately for different litter fractions. Samples from Hyytiälä during 1999–2005 were analyzed by an elemental CN analyzer (LECO, Leco Corporation, St. Joseph, MI, USA). Samples from Hyytiää during 2006–2010 and from Speulderbos were analyzed by a Vario MAX CNS elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

2.4 Meteorological data

The meteorological parameters measured and devices employed can be seen in Table 2.

Mean wind speed was calculated averaging 30 min data. Wind gusts were reported when the wind speed reached 8.2 m s^{-1} and the variability of the wind from highest point to lowest was more than 4.6 $m s^{-1}$. Beaufort wind speed scale was used for qualitative identification of wind gusts: 8.0–10.7, 10.8–13.8; 13.9–17.1, 17.2–20.7; and 20.8–24.5 m s^{-1}.

2.5 Data analysis

Data of litterfall and meteorological parameters (2006–2011) were calculated by regular periods of one calendar month each: the dates of litter collection period did not correspond to calendar months, therefore, the value of daily litterfall rate measured at the end of each period was considered the same for every day of the collection period; an average daily value was then calculated for each calendar

Table 2 Meteorological devices used at the two study sites

Meteorological devices	Hyytiälä	Speulderbos
Air temperature	(16.8 m) ventilated and shielded Pt-100 sensor (platinum resistance thermometers)	(45 m) Vaisala WXT510 and Thermocouple E-type (Vaisala Oyj., Helsinki, Finland)
Wind speed	(23 m) sonic anemometer Solent 1012R2 (Gill Instruments, Lymington, UK)	(46 m) sonic anemometer 2D (Gill instruments Ltd., Lymington, UK)
Global radiation	(18 m) Reemann TP 3 pyranometer (Astrodata, Tõravere, Estonia)	(46 m) CNR-1 Net radiometer (Kipp & Zonen, Delft, The Netherlands)
Precipitation	(2 m) ARG-100 tipping bucket rain gauge (Institute of Hydrology, Wallingford, UK)	(47 m) Custom-made tipping bucket & Vaisala WXT510 (Vaisala Oyj, Helsinki, Finland)
Soil temperature	(−5 cm) five silicon temperature sensors KTY81-110 (Philips Semiconductors, Eindhoven, the Netherlands)	(−5 cm) NTC thermistors (*Murata Machinery Ltd., Kyoto, Japan)*
Soil water content (0–30 cm)	TDR-method with unbalanced steel probes 1502C (Tektronix Inc., Redmond, USA)	Water content reflectometer CS-616 (Campbell scientific Inc., Logan, USA)
Average snow cover	Daily measurements	Daily measurements

month as the arithmetic mean of all daily values for the month considered, thus 12 values per year. This transformation resulted in the standardization of number of data points along the year, and eliminated differences between both species in the litter collection dates. It permitted to compare statistically both datasets, to make year-to-year comparisons, and improved visual understanding of graphs. Outliers were defined using 1.5 times the difference between the third and first quartiles, as described in Morillas et al. (2012).

The leaf nitrogen resorption proficiency (NRP) was defined as the minimum N concentration per unit of leaf mass found in senescent leaves (Killingbeck 1996).

2.6 Numerical analysis

The spatial variability in the litterfall collected during the study period was assessed with the calculation of the coefficient of variation (CV) between litter traps: the cumulative mass collected over the whole study period (in gramme) was calculated for each litter trap. The standard deviation of the litter trap collection values was divided by their arithmetical mean.

C and N production rate in litterfall (gramme of C and milligramme of N per square metre per day) were calculated by multiplying the C or N content (gramme of C and milligramme of N per gramme of biomass) of each litter fraction for a given collection period by the respective litterfall rate (gramme per square metre per day). The values of C and N production rates for each collection period were subsequently transformed to calendar monthly values as explained in the chapter 2.5, thus obtaining one value per calendar month.

For Hyytiälä site, the identification of two seasons in monthly C and N input to the forest floor (also called C and N production rate in litterfall) was found after a paired *t* test: monthly values during the study period were grouped into two seasons (colder season vs. warmer season, covering the whole year period) throughout the possibilities (varying the season length and the starting month), the option which produced the highest significance (lowest P value) in a t tests was chosen as the most appropriate way to study the two seasons in each study site.

The inter-annual variations in Scots pine litterfall were assessed. The calculation of the average annual litter production in a given year is explained in Eq. 1, where: P is the litter collected (megagramme per hectare per year) in a given year Y_i, and P_{av} is the average annual litter production from Y_0 until Y_N.

$$P_{av} = \frac{\sum_{i=0}^{N} P(Y_i)}{N+1} \quad (1)$$

Normality tests (Shapiro–Wilk) were performed to yearly data prior to analysis (Morillas et al. 2012), which resulted in a reasonable number of rejected null hypotheses. Nevertheless, we used parametric tests to analyze the data. Statistical comparisons between stands and between years were performed by two-sample t tests and analysis of variance (ANOVA).

3 Results

3.1 Climatic differences

Maximum air and soil temperatures were similar at both sites (Fig. 1). Hyytiälä presented a wider range of air temperatures, usually below 0 °C from November to December, while the winters at Speulderbos were warmer (Fig. 1). Soil

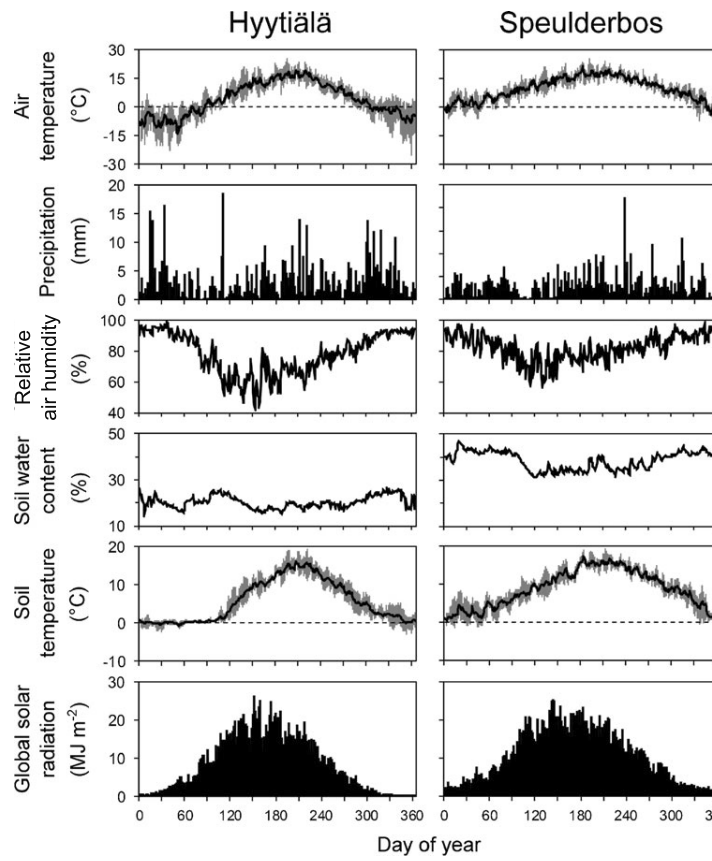

Fig. 1 Daily averages of climatic data at Hyytiälä (Finland) and Speulderbos (the Netherlands) during the periods August 2007–December 2010 and June 2007–December 2010, respectively. Air temperature was measured at 16.8 (Hyytiälä) and 45 m (Speulderbos), volumetric soil water content at 0–30-cm depth and soil temperature at 5-cm depth. Gray area in air and soil temperature plots covers the range between daily maximum and minimum values

temperature was stable during winter time due to the snow cover at Hyytiälä; at Speulderbos, winter time was warmer (Fig. 1). Precipitation was fairly uniformly distributed along the year in both sites (Fig. 1). Relative air humidity during the growing season was lower at Hyytiälä (Fig. 1). Soil water content was relatively constant during the season in both sites, and was higher at Speulderbos (Fig. 1). Annual global radiation received was similar at both sites, being slightly higher at Speulderbos (7.97 $MJm^{-2}day^{-1}$ in Hyytiälä vs. 9.60 $MJm^{-2}day^{-1}$ in Speulderbos).

3.2 Litter collection characteristics

The spatial variability in the litterfall collected returned as a result a CV of 21.5 % in the more open and shorter Scots pine stand in Hyytiälä, and 4.7 % in the more dense and taller Douglas fir stand in Speulderbos.

Inter-annual coefficient of variation of annual litter dry mass production during the study period was 26 % for Scots pine, and 13.3 % for Douglas fir.

The length of the study period importantly affects the assessment of the stand-level inter-annual average (Fig. 2). In Hyytiälä, the difference between the annual values and the long time period mean were great in some years (Fig. 2a). Two examples of that difference occurred in 2004 and in 2011, where the actual litterfall measurements were 35 % smaller than the full study period average of six and thirteen years, respectively (Fig. 2b). In contrast, in year 2002, the litterfall measured was 35 % greater than the average estimated for the last 4 years (Fig. 2b). Furthermore, this value was 72 % greater than the average from 1999 to 2011 period. If this value would be considered as an outlier, the new annual litterfall average for the site would change only by 6 %.

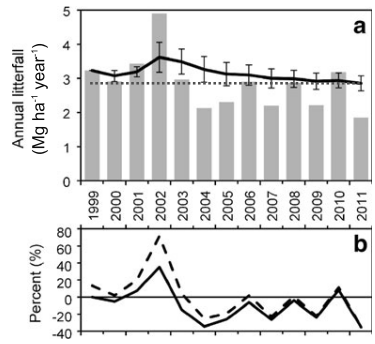

Fig. 2 Variations in annual litterfall in Hyytiälä in Scots pine-dominated conifer forest through years 1999–2011. In **a**, the *grey bars* represent the actual dry mass of litterfall collected by the litter traps during the entire year, whereas the *line* shows the averaged (average ± SE) annual litter production (P_{av}) from 1999 until the given year Y_i, (Eq. 1). The *dotted line* shows the mean annual litterfal value for the whole study period (2.86 Mgha^{-1}year^{-1}) In **b**, the *solid line* shows the difference in percentage between the actual collected litter and the annual litterfall average calculated since 1999 until the year Y_i. The *dashed line* represents the difference in percentage between the actual collected litter and the 13-year period litterfall average

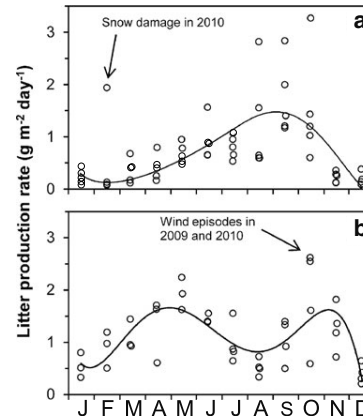

Fig. 3 Monthly average litter production rate in Hyytiälä (**a**) for 2006–2010 and Speulderbos (**b**) for July 2007 to December 2010. The *trendlines* correspond to third-order (**a**) and fifth-order (**b**) polynomial regressions with r^2 values of 0.46 (**a**) and 0.47 (**b**) ($P<0.001$ for both). The data point of high litterfall in February 2010 (**a**) corresponded to snow damage in Scots pine trees, the two observations of high litterfall in October 2009 and 2010 (**b**) corresponded to storms with exceptionally high wind speed episodes

3.3 Overall patterns in litter production

The average (± SE) annual fine litter dry mass production for the study period was 2.86±0.22 Mgha^{-1}year^{-1} in the Scots pine stand in Hyytiälä (1999–2011) and 4.34±0.33 Mgha^{-1} year^{-1} in the Douglas fir stand in Speulderbos (2008–2010). Fine litter production rates in Scots pine showed an unimodal distribution with a maximum in autumn (2.82 gm^{-2}day^{-1} in August 2006, 1.43 gm^{-2}day^{-1} in September 2007, 3.27 gm^{-2} day^{-1} in October 2008, 2.84 gm^{-2}day^{-1} in September 2009, and 1.99 gm^{-2}day^{-1} in September 2010) (Fig. 3a). During snow cover period (November–March) a small amount of litter was produced. Minimum Scots pine litterfall rates were reached in months between December to February, and ranged from 0.078 to 0.127 gm^{-2}day^{-1}. Douglas fir (Fig. 3b) showed a more likely bimodal distribution. Litterfall was more evenly distributed during the year, and maximum peaks were less pronounced than as for Scots pine because of the less severe winter period in Speulderbos; nevertheless, litter was mainly produced from April to May and from October to November. Annual maximum values of litter production were 1.93 and 1.61 gm^{-2}day^{-1} in May and October 2008, 2.24 and 2.54 gm^{-2}day^{-1} in May and October 2009, 1.71 and 2.62 gm^{-2}day^{-1} in April and October 2010 for these two peaks (Fig. 3b). Annual minimum values were reached in December and January, with values from 0.21 to 0.33 gm^{-2}day^{-1}.

3.4 Fractional composition of litter

Fractional litterfall compositions resulted in significantly different dynamics between the litter fractions (needle cones, twigs, bark, rest, and cones and seeds) in both species (paired-samples *t* tests, $P<0.001$); using monthly time series of litterfall (2006–2010 for Scots pine and July 2007–December 2010 for Douglas fir). Douglas fir produced a greater relative amount of needle litter (inter-annual average ± SE=78.929±0.011 %) than Scots pine (60.952±0.035 %).

There was a strong relationship between the leaf litterfall fraction and the total litterfall (Table 3).

Scots pine litter production rates by fraction followed typical unimodal distributions with absolute maxima in September (needle, Fig. 4a), May (twigs, Fig. 4b), June and July (cones and seeds, Fig. 4c; bark and rest, Fig. 4d); while the maximum total litterfall during the 2006 to 2010 period was reached in September (Fig. 3a). In spring, Scots pine bark was an important component of the total amount produced. In late summer and early autumn, needle fraction predominated. In May 2006 and February 2010, there were peaks of twig litter, and in June and July 2007, peaks of cones and seeds.

Douglas fir needle litterfall (Fig. 4e) peaked in May and October, twig fraction (Fig. 4f) in March and October, and cones and seeds (Fig. 4g) in June. Bark (Fig. 4h) litterfall rates were more distributed along the year, not showing a specific pattern.

The minimum annual litterfall rates were mainly constituted of needle litterfall in Douglas fir (82±10 % in average). However, in Scots pine, at the litterfall minima, the needles represented only the 40±17 % (twigs 25±8 % and bark 21± 10 %).

3.5 Inter-annual and seasonal differences in litter production in relation to climatic drivers and extreme weather events

Monthly data of climatic variables (global radiation, air temperature, soil temperature, and soil water content) did not significantly correlate ($P>0.05$) with the total litterfall, needle litterfall, and C and N production rate in both sites.

In Hyytiälä, horizontal wind speed recorded from 1999 to 2011 was rarely higher than 8 ms^{-1}, and maximum wind gusts over the period were lower than 21 ms^{-1}. No significant relationships were found between wind speed and litterfall production ($P>0.5$). The number of hours per month of 30-min averaged high wind speed data (wind speed 8.0–10.7 and 10.8–13.8 ms^{-1}) did not show correlation to monthly amount of litterfall ($P>0.05$).

In Speulderbos, wind gusts recorded from July to March had an average speed of 18.8±3.6 ms^{-1} with a maximum of 30.3 m s^{-1}. The maximum wind gust speed data correlated positively to total litterfall ($r^2=0.40$***), and with needle ($r^2=0.32$***) and twig ($r^2=0.38$***) production rates. The high values 2.544±0.041 and 2.6±1.0 g litter $m^{-2}day^{-1}$ in October for 2009 and 2010, respectively (Fig. 4b), could have been caused by storm episodes at Speulderbos. We identified half-hour averaged wind speed of 8.0–9.1 ms^{-1} during several hours with maximum wind gusts of 20 ms^{-1}. The needle litterfall in those episodes was 44 % higher than in the same period of year 2008. The litterfall for those months contained higher non-needle fractions (bark 1.0 %, twigs 13.2 %, and cones and seeds 2.7 %) compared with the same periods in years 2007 and 2008 (bark 0.2, twigs 6.0 %, and cones and seeds 1.3 %). Finally, the litter produced during storm episodes increased the annual litterfall by 6.7 % in 2009 and 6.8 % in 2010, compared to October 2008.

At Hyytiälä site, early frost events and unusual low temperatures were identified in September 1999, October 2000, October 2008 and September 2009, and these events were possibly related to high litter production rates. Also, severe snow damage to the trees was recorded during winters 2009–2010 and 2010–2011. In 2009–2010, about 7 % of the basal area was lost, increasing the litterfall (Fig. 3a). This point was considered an outlier and not taken in account for the trend line in Fig. 3a.

In Hyytiälä, extremely low precipitation values were associated with extreme litter production rates. The year 2002 was exceptionally dry (535 mm), specially in summer time, and annual litterfall was extraordinarily high (4.90 Mg $ha^{-1}year^{-1}$; Fig. 2). These values of precipitation and litterfall were distribution outliers (atypical points) in the 13-years distribution from 1999 to 2011. Scots pine annual needle litterfall peak in year 2006 occurred in August, earlier than in the subsequent years, probably related to drought effect observed during the year 2006. A peak of 1.938 g litter $m^{-2}day^{-1}$ constituted mainly by twigs and needles was observed in February 2010 (Fig. 3a), possibly related to the combined effect of the snow load on the branches and high 24-h averaged wind speed of 5.72 ms^{-1} with associated wind gusts and maximum 30-min average wind speed of 7.94 m s^{-1}.

3.6 Carbon and nitrogen inputs to the forest floor

Annual C and N inputs to soil were mainly driven by needle litterfall (Table 4). The N annual input through litterfall to Hyytiälä stand soil (15.9±1.5 kg N $ha^{-1}year^{-1}$) was much lower than in Speulderbos stand (75±8 kg N $ha^{-1}year^{-1}$), if compared with the C annual input (1,430±110 and 2,300± 180 kg C $ha^{-1}year^{-1}$, respectively) (Table 4).

In Hyytiälä, there were two differentiated seasons regarding the C and N inputs to soil through litterfall: May–October and November–April (paired-samples t tests, $P<0.001$). Average (±SE) C input rates to soil in Hyytiälä were 0.574±0.039 g C $m^{-2}day^{-1}$ from May to October and 0.204 ±0.020 g C $m^{-2}day^{-1}$ from November to April. N input rates for these seasons were 6.48±0.49 and 1.969± 0.037 mg N $m^{-2}day^{-1}$, respectively.

The high rate of twig litterfall of Scots pine exhibited in February 2010 (Fig. 3a) did not remarkably affect the C or N litter contents because of the similar element percentages to those for the needles. In contrast, the high portion of cones and seeds in June and July 2007 in litter (40 % of total litterfall) lowered the total litter N content by 27 % compared with June and July averages for years 2006, 2008 and 2009. Difference in cone and seed production did not affect C content.

The litter C and N content did not show any significant Pearson's linear relationship with the litterfall rate in any of both species ($r^2<0.012$). But the litterfall C and N input to soil rates were highly correlated between them, and with the total litterfall and needle litterfall (Table 3).

C content was similar along the litter fractions in both species. In Scots pine, the rest fraction was the richest in N content, followed by the needles (Table 4). In Douglas fir, N content differed between fractions (Table 4). From a temporal view, litter N content fluctuated more along the year in Douglas fir than in Scots pine, which also contained less N (Fig. 5). Douglas fir litter N content in October 2009 and 2010 (1.82 and 1.84 %, respectively) was 16 % higher than in October 2007 and 2008 (1.50 and 1.66 %, respectively), while carbon content was similar (Fig. 5d), and consequently, C/N

Table 3 Equations of linear models ($y=ax+b$) fitted to litterfall characteristics relationships: y correspond to the traits in the second column, x correspond to the titles of the following columns. Scots pine (Jan 2006–Dec 2010) and Douglas fir (Jul 2007–Dec 2010)

Species	Litter traits	LF_{tot}	LF_{nee}	LF_C
Scots pine	LF_{nee}	$0.8895x-0.2023$		
	LF_C	$0.5094x-0.0042$	$0.5190x+0.1355$	
	LF_N	$0.0057x-0.0002$	$0.0061x+0.0012$	$0.0113x-0.0002$
Douglas fir	LF_{nee}	$0.8195x-0.0360$		
	LF_C	$0.5325x+0.0001$	$0.6035x+0.0632$	
	LF_N	$0.0176x-0.0003$	$0.0200x+0.0018$	$0.0328x-0.0002$

LF_{tot} and LF_{nee} are total litterfall and needle litterfall rates (gramme per day); LF_C and LF_N are carbon and nitrogen production rates (gramme per square metre per day)

Parameters are monthly-averaged. The linear regressions are calculated with monthly values ($n=60$ for Scots pine; $n=42$ for Douglas fir)

Pearson's linear correlation coefficients (r^2) are from 0.839 to 0.999, all coefficients are significant at $P<0.001$ (two-tailed)

ratio 14 % smaller (29.3 in 2009 and 28.9 in 2010; Fig. 5f). As a result, the average soil C input rate was 135 % greater (Fig. 6c) in October 2009 and 2010 compared with the same period in 2007 and 2008, whereas the average N input rate average was 167 % greater (0.046 g N $m^{-2}day^{-1}$ in 2009 and 0.048 g N $m^{-2}day^{-1}$ in 2010; Fig. 6d).

Generally, there were two periods during the year at the Douglas fir site when C and N were released to the topsoil from the canopy litter (Fig. 6c, d), while there was only one period at the Finnish site (Fig. 6a, b). Litter C production rate (Fig. 6a, c) followed the same pattern as the total litter production rate (Fig. 3a, b), reflecting the circumstance that litter C concentration almost did not vary during the year (Fig. 5a, d). Despite, Scots pine litter C (50.74±0.27 %; Fig. 5a) and N content (0.642±0.025 %; Fig. 5b) had maxima in September. Litter was richer in N with a minimum C/N ratio in September (79.1±2.8; Fig. 5c), where the smallest yearly standard deviation was found.

4 Discussion

4.1 Litterfall characteristics

At the Hyytiälä site, the inter-annual variations in litter production emphasize the importance of long study periods (Fig. 2). Measurements repeated on the same trees revealed a fluctuating pattern (Fig.2a): after one period with higher canopy litterfall succeeded another period with smaller amount and vice versa. These fluctuations can be consequences of annual irregularities in the climate, which can create a higher needle loss with nutritional problems for the

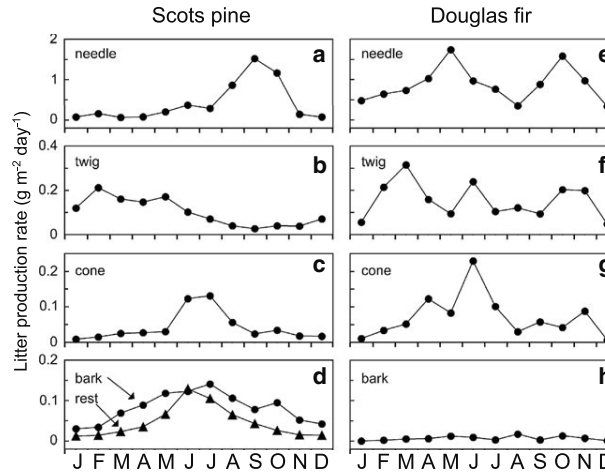

Fig. 4 Annual dynamics of litterfall fractions for the periods 1999 to 2011 in Scots pine (**a**, **b**, **c**, **d**), and July 2007 to December 2010 in Douglas fir (**e**, **f**, **g**, **h**)

Table 4 Average (±SE) litter mass fractionation, litter C and N contents, and annual inputs of biomass, C and N to the forest soil in Hyytiälä (Jan 1999–Dec 2010) and in Speulderbos (Jan 2008–Dec 2010)

Species	Litter fraction	Mass percentage (%)	C content (%)	N content (%)	Annual biomass soil input (kg ha^{-1} year^{-1})	Annual C soil input (kg C ha^{-1} year^{-1})	Annual N soil input (kg N ha^{-1} year^{-1})
Scots pine	Needle	61±8	51.16±0.19	0.663±0.038	1,600±120	800±70	9.1±1.3
	Twigs	14±6	49.40±0.15	0.454±0.047	580±190	180±90	2.3±0.9
	Bark	11.8±2.2	49.20±0.40	0.416±0.016	290±11	141±6	1.31±0.14
	Cones and seeds	7±5	47.07±0.26	0.228±0.031	200±29	93±13	0.70±0.16
	Rest	6.5±0.8	50.32±0.25	1.278±0.049	166±7	83±4	2.05±0.11
	Total	100	50.27±0.24	0.601±0.036	2,860±220	1,430±110	15.9±1.5
Douglas fir	Needle	78.9±1.9	53.19±0.27	1.80±0.23	3,420±310	1,820±80	62±7
	Twigs	14.33±0.39	53.0±0.6	1.07±0.37	620±5	330±29	6.6±1.8
	Bark	0.45±0.13	52.8±0.9	1.055±0.041	20±5	10.6±4.2	0.21±0.05
	Cones and seeds	6.3±2.1	53.2±2.6	2.0±1.4	265±32	140±80	5.3±4.5
	Total	100	53.1±1.1	1.7±0.5	4,340±330	2,300±180	75±8

The mass fractions and elemental contents among the litter components within species were compared by one-way ANOVA, showing significant differences ($P<0.001$)

The averages shown in the table are calculated from 3-months intervals collected litter during 1999–2005 and monthly intervals during 2006–2010

trees in the subsequent period; or longer-term variations like drought years period or plagues. As we cannot control every climatic and physiological parameters, it is important to identify whether the data outliers were produced by sampling errors or by actual variations in litterfall. Outliers removal, numerical transformations or other data processing

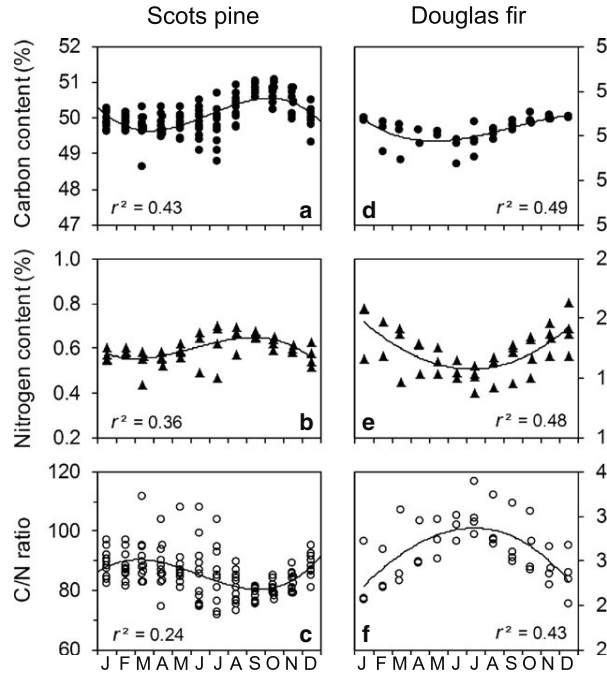

Fig. 5 Litterfall quality (C and N content, and C/N ratio) of Scots pine (1999–2009) in Hyytiälä and Douglas fir (July 2007 to December 2010) in Speulderbos. Data correspond to monthly averages during the study period. Data were fitted by third-order polynomial regressions; all are significant at $P<0.001$

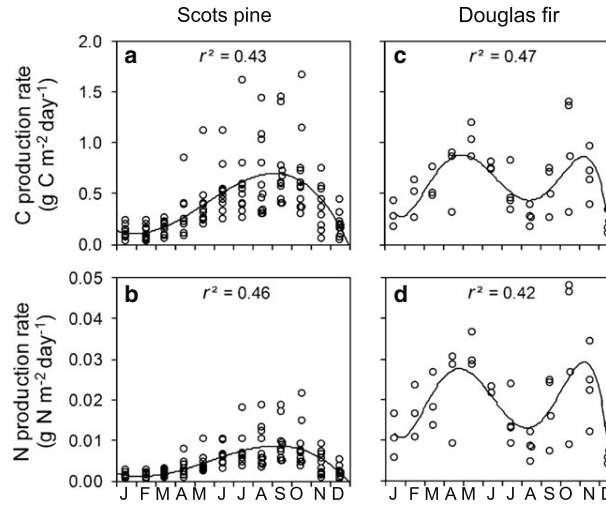

Fig. 6 Carbon and nitrogen production rate in litterfall of Scots pine (1999–2009) in Hyytiälä and Douglas fir (July 2007 to December 2010) in Speulderbos. Data of Scots pine were fitted by third-order polynomial regression and those in Douglas fir by fifth-order polynomial regressions

methods must be done with extreme caution because the results can be modified drastically and interesting litterfall events might be ignored.

The Eq. 1 showed that the increasing number of data yields to a less deviated mean if compared to the mean over the years (Fig. 2b). The Eq. 1 permitted to highlight the biased viewpoint of short-term studies on the annual average litterfall. The annual litterfall of a stand is far from being a steady value (Kouki and Hokkanen 1992; Ranger et al. 2003), it fluctuates. Depending of the length of the study period, scientists may calculate different average values for annual litterfall in the same stand. As an example, Ranger et al. (2003) reported big variations in Douglas fir annual litterfall (from ca. 2.5 to 6 Mg ha^{-1} $year^{-1}$) during a 7-year study in France. Fig. 2b showed the importance of long-term studies concerning litterfall. However, climatic factors, extreme weather events, stand growth, or forest management practices like thinning will play an important role on the litterfall rates. This is why litterfall averages of long-term studies must be used carefully. Understanding the processes which affected them and the status of the stand can be more important than the litterfall average itself. In the other hand, the average annual litterfall in Speulderbos can be taken only as a first-stage estimate; this is why this article studies more the intra-annual characteristics for the Speulderbos stand.

Although being both monoculture conifer forests and having installed a double amount of litter traps of similar size in Hyytiälä, CV between litter traps was relatively high. The Scots pine stand was partially thinned during the experiment, which can explain the spatial differences in litterfall. Also, the CV values can reflect the more open canopy of the Scots pine stand in contrast to the Douglas fir stand, with 6.5 $m^2 m^{-2}$ (all-sided) LAI (Kolari et al. 2009) and 7.8 to 10.5 $m^2 m^{-2}$ (projected) (Van Wijk et al. 2000), respectively. Nonetheless, McShane et al. (1983) found a high CV between litter traps while studying a Douglas fir stand. In addition, despite a similar twig mass was produced in both stands, the bigger pine twig size enhanced the CV, producing less single pieces with higher mass each and higher difference in the collected litterfall between litter traps.

4.2 Correlations of litter production with environmental drivers and effects of extreme events

Climatic parameters had an important effect on litterfall dynamics. Extreme values were especially important to understand unusually high peaks of litterfall production (i.e. drought effect in 2002 and 2006, early frosts and snow damage at Hyytiälä, and wind gusts effect on Speulderbos).

At Hyytiälä site, wind speed did not reach as high values as at Speulderbos. Data showed that Douglas fir litterfall was affected by high wind speeds (maximum wind gusts). In addition to wind-influenced litterfall, wind may damage the internal structures of the branches. This effect decreases the tree growth rate and needle production in the subsequent years, which will decrease the needle litter production in a short term. Nevertheless, damaged twigs are shed later on. In conclusion, these damages can create a biased disturbance in the litterfall rates of stands frequently affected by phenomena such as wind damage, etc.

Premature needle abscission can be responsible for lower resorption rates. The early frosts that occurred at Hyytiälä

had a direct effect on litter N content enhancement (Fig. 6b), associated to the highest values of minimum annual needle N concentration. Also, the early frosts in 2008 and 2009 could have affected the secondary growth of Scots pine, finding less cone production in the subsequent years.

Table 3 showed that the litterfall production rates and the C and N soil input rates were correlated. Despite isolated events of high litterfall and C and N soil input, the linear models shown in Table 3 were highly significant.

4.3 Litter production and fractional composition of litter

Similar values for annual Scots pine litter production have been reported by Berg et al. (1999) and Starr et al. (2005) in stands with similar age in similar latitudes. In the natural environment of Douglas fir, McShane et al. (1983) found similar annual litterfall values in a stand of similar age. Also, Ranger et al. (2003) reported similar values for a 40-year stand in France. In Speulderbos, when the stand was 35 years old, Koopmans et al. (1996) calculated a N input to soil of 51 kg N $year^{-1}$ (ca. 0.014 g N $m^{-2}day^{-1}$), and our results give an input of 0.019 g N $m^{-2}day^{-1}$. This increase can be interpreted as a result of the stand growth (Ukonmaanaho et al. 2008).

Our study demonstrates that sorting the litterfall in fractions allows to gaining important insight into the determinants of inter-annual and seasonal variations in litter production rate and chemistry. Litter production rates for different litter components were statistically different. This is important as the fractional composition of litter directly determines which type of litter is added to the forest soil in every period of the year. Regarding the physical characteristics (toughness, shape, or amount) and chemical composition of the litterfall, and the meteorological and biological status of the soil, it influences the litter decomposability and nutrient release to the soil.

The maximum litterfall rates in summer of cones at both stands (Fig. 4c, g), bark and rest, may suggest that trees shed the materials *unused* during the growth season. The stem and branches could increase the bark litterfall in Scots pine as they grow and increase their diameter. The resin droplets of the rest fraction in Scots pine are secondary products of tree metabolism, and principally produced in that season. And mature cones could be liberated in summer in favor of higher priority processes like maintaining photosynthetic needles.

4.4 Seasonality of leaf litterfall

Deciduous trees in the climates of this study (i.e. oak, beech, maple and birch) have a strategy of building one-season leaves, which grow and senesce in some months. It is in autumn, where conditions get unfavorable for the net primary production (colder temperatures and less light), that leaves senesce and fall to the topsoil, generating the annual maximum N input to these soils (excluding sites with high atmospheric N deposition). Situation in evergreen conifers (i.e. the species of this study, pine trees and firs) is different: they support several cohorts of leaves on their canopy lasting several years. The leaf longevity of Scots pine and Douglas fir at the study sites is about 2 to 3 years (Wang et al. 2012). Leaf longevity in these species is variable inside the same tree: new leaves are produced in spring and old leaves are shed during the whole year, although there are leaf litter production peaks. Evergreen broad-leaved Mediterranean species (i.e. cork oak, olive, *Arbutus* and *Nerium oleander*) conserve leaves for more than one growing season, but the shedding of leaves occurs during the hot and dry summer months.

Despite similar physiological characteristics in both our species, litterfall seasonality was different (Fig. 3). During winter in Hyytiälä, the needles are in dormant phase and it is beneficial for the plants to drop the needles right before this phase. Therefore leaf litter production in winter is normally small and presumably caused mainly by physical damage to the trees. At Speulderbos, which has warmer climate, the rate of litter production processes only decreased during winter (Fig. 3). Speulderbos conditions generated an overall higher leaf litter production and it presented higher leaf area index, helped by the higher and moderate temperatures by the latitude and maritime influences.

4.5 Litter chemistry

Nutrients lost in litterfall are a cost to a tree, as they have to be substituted to maintain primary productivity (Lin et al. 2003). Boreal soils are typically N limited (Priha and Smolander 1999); climate presents lower temperatures and longer and colder winters. Our results of Scots pine needle N content are in concordance with Ukonmaanaho et al. (2008), which reported a mean of 0.49 % in senescent needles in Finnish forests. The narrow fluctuation range and low annual average of Scots pine litter N concentration observed is typical for a species adapted to N limited ecosystem, even lower than the N content reported by Niinemets et al. (2001) (0.86±0.12 %) in an infertile site in Estonia. Concerning Douglas fir, Turner and Olson (1976) reported a value of 0.57 % for a 42-year-old Western Washington stand. Ranger et al. (2003) found litter N concentrations of 0.91–1.38 % in a 7-year study in France, with a seasonal dynamic similar to our study. Our values are still higher possibly because of the chronic N deposition, which could have increased needle N concentration (Turner 1977). Furthermore, Simpson et al. (2006) found Speulderbos site to be particularly affected by N deposition, while Hyytiälä belonged to the less affected areas in Europe.

4.6 Nutrient resorption efficiency and nutrient losses by litter

According to Killingbeck (1996), leaf litter N concentrations observed during the year and its minimum (NRP) indicated very good capacity for senescent leaf nutrient resorption (NRP<0.7 % N) in the Scots pine stand (Fig. 5b). This is typical of a species adapted to low-fertility soils (Pensa and Sellin 2003) and high-stress environmental conditions. Similar NRP values (0.50 and 0.52 %) were reported by Killingbeck (1996), and even lower NRP values (0.44 % N) in Scots pine grown on podzols in Estonia were found by Pensa and Sellin (2003). In contrast, Douglas fir stand performed in every year an incomplete N resorption (NRP<1 % N), but its favorable site conditions regarding soil N availability, higher temperature and light may compensate this inefficacy, and help the stand reaching high growth rates per year. A most exhaustive sampling of senescent needles during nutrient resorption period might reveal a most accurate NRP value.

The storm episodes in 2009 and 2010 in Speulderbos led to an increase in annual litterfall. The loss of cones, the loss of twigs as support structures for potential new needles and the loss of needles itself during the nutrient retranslocation period resulted in an incomplete nitrogen resorption, if compared with previous years NRP values (2007 and 2008).

5 Conclusion

These data collectively demonstrated that there were large inter- and intra-annual variations in canopy litterfall. Longer study period will produce better result on the site litterfall average calculation. Inter-annual fluctuations in litterfall may lead to biased values in the annual litterfall estimates if the study period is short.

Weather conditions played a big role on litterfall. These importantly altered litter production and litter quality. Both species had different meteorological conditions and were affected differently by meteorological events. Scots pine litterfall in Hyytiälä was affected by annual low precipitation, early frosts and snow load. Douglas fir litterfall was mostly affected by wind gusts. A principle of effect of unexpected premature leaf shedding on leaf nitrogen resorption efficiency was found in this study; senescent leaf minimum N concentration was found to increase in Douglas fir after significantly high leaf fall events, if compared with other years. This particular subject needs further research.

When studying litterfall, researchers must pay attention to the climatic variables and single events which could affect it. Litterfall rates usually vary largely from year to year. More studies about the causes of these differences are needed to properly model the nutrient cycling in forests.

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III

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LITTERFALL DYNAMICS AND VOC EMISSIONS FROM SOIL LITTER LAYER
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Volatile compounds emission from canopy fine litterfall in a hemiboreal mixed forest at Järvselja

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The seasonal distribution of biogenic volatile organic compounds (BVOC) emissions from canopy fine litterfall was investigated over a period of two years.

Three stands of a hemiboreal mixed forest were studied. The stands presented different dominant tree species: (1) Norway spruce, (2) Scots pine, and (3) Silver birch and Downy birch. The litterfall was monthly collected in litter traps. The BVOC emission of litter was sampled by placing the litter into a glass jar equipped with a vent tube and pumping the head space air through a VOC adsorbing tube (carbotrap). Adsorbed BVOCs were analyzed in a GC-MS. Fifteen compounds were quantified.

Seasonal differences in the total emission of BVOCs were found, defined by a maximum in summer and a minimum in autumn and winter. During summer months, litter emissions were dominated by limonene, α-pinene, camphene and 3-carene in the three litter types, accounting for 70-75 % of total BVOC emitted in June. 3-Carene, α-pinene and β-pinene were the main compounds emitted during winter time, accounting for 50-60 % of total BVOC emitted in January.

Stand to stand differences were assessed. The spruce and birch dominated stands showed more similarities in their BVOC emission pattern if compared to the pine dominated stand. Together with the litterfall data, an estimation of the annual total BVOC emitted by the soil litter layer is presented for each stand type.

IV

Noe SM, Kimmel V, Hüve K, Copolovici L, **Portillo-Estrada M,** Püttsepp Ü, Jõgiste K, Niinemets Ü, Hörtnagl L, Wohlfahrt G (2011) ECOSYSTEM-SCALE BIOSPHERE–ATMOSPHERE INTERACTIONS OF A HEMIBOREAL MIXED FOREST STAND AT JÄRVSELJA, ESTONIA Forest Ecology and Management 262:71–81

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Ecosystem-scale biosphere–atmosphere interactions of a hemiboreal mixed forest stand at Järvselja, Estonia

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ABSTRACT

During two measurement campaigns, from August to September 2008 and 2009, we quantified the major ecosystem fluxes in a hemiboreal forest ecosystem in Järvselja, Estonia. The main aim of this study was to separate the ecosystem flux components and gain insight into the performance of a multi-species multi-layered tree stand. Carbon dioxide and water vapor fluxes were measured using the eddy covariance method above and below the canopy in conjunction with the microclimate. Leaf and soil contributions were quantified separately by cuvette and chamber measurements, including fluxes of carbon dioxide, water vapor, nitrogen oxides, nitrous oxide, methane, ozone, sulfur dioxide, and biogenic volatile organic compounds (isoprene and monoterpenes). The latter have been as well characterized for monoterpenes in detail. Based on measured atmospheric trace gas concentrations, the flux tower site can be characterized as remote and rural with low anthropogenic disturbances.

Our results presented here encourage future experimental efforts to be directed towards year round integrated biosphere–atmosphere measurements and development of process-oriented models of forest–atmosphere exchange taking the special case of a multi-layered and multi-species tree stand into account. As climate change likely leads to spatial extension of hemiboreal forest ecosystems a deep understanding of the processes and interactions therein is needed to foster management and mitigation strategies.

1. Introduction

Forest ecosystems are a major part of the biosphere and control as such land surface–atmosphere interactions. They influence atmospheric composition and climate significantly because of their importance as sources and sinks of trace gases and energy on both, local and regional scales (Högberg, 2007; Magnani et al., 2007; Misson et al., 2007). To better understand both, how forest ecosystems will respond to changes in climate and how they will feed back to the atmosphere, we need to gain quantitative insight into the energy and matter fluxes between the biosphere and atmosphere.

Forests themselves form complex ecosystems, exhibiting a great variability in their composition and structure in horizontal and vertical directions. Major fluxes of carbon dioxide, methane and water vapor are exchanged between soil, plants and the atmosphere at several distinct layers. Those layers are characterized by very different functional properties and they also differ in their flux contributions (Baldocchi, 1997; Misson et al., 2007).

Reactive trace gases such as ozone or nitrogen oxides (NO_X) and sulfuric compounds for example represent abiotic stressors to the plants in the forest ecosystem, causing reductions in biomass production (Wallin et al., 1990; Laurence et al., 2001; Manning et al., 2003). Together with biogenic volatile organic compounds (BVOC) that are emitted by many plant species to substantial amounts, these reactive trace gases determine the local, regional and global air quality. Their reaction products play a major role in the formation and growth of aerosol particles, ozone and organic acids (Atkinson and Arey, 2003; Bonn et al., 2008; Hewitt et al., 2009; Mentel et al., 2009).

Hemiboreal forests, located in the transition zone between boreal and temperate forest biomes, are characterized by mixed stands of both coniferous and deciduous tree species, where deciduous species can dominate the initial stages of succession, being replaced by coniferous species at later stages of succession. Especially in mixed stands, the presence of deciduous tree species exerts a great variability on the forest microclimate, canopy shape and

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density by closing the canopy in spring and opening it up again in autumn when the leaves drop off.

Typically, the presence of multiple leaf layers in the canopy leads to gradients of environmental variables such as light, temperature, wind speed and turbulent mixing. These gradients in turn affect the magnitude of the ecosystem fluxes throughout the canopy. Furthermore, differences in energy availability and mixing properties of the air below and above canopy result in within-canopy variation in concentrations of carbon dioxide and reactive trace gases impacting on the growth and biomass production of the trees differently at different canopy layers.

So far, integrated forest ecosystem scale studies, covering leaf, soil and ecosystem fluxes of reactive and non-reactive trace gases have not yet been conducted for hemiboreal forest ecosystems in Estonia. Our goal was to assess a first overview on fluxes and concentrations of carbon dioxide, water vapor and reactive trace gases under the influence of a hemiboreal forest ecosystem. Specifically the occurrence of a major and suppressed tree layer and consequences thereof on the fluxes and concentrations are of particular interest. Therefore, we present below and above canopy eddy covariance fluxes, leaf level net photosynthesis rates, soil trace gas fluxes, within and above-canopy trace gas concentrations, and BVOC emissions measured during two field campaigns from August to September 2008 and 2009 at the Experimental Forestry Station in Järvselja, Estonia.

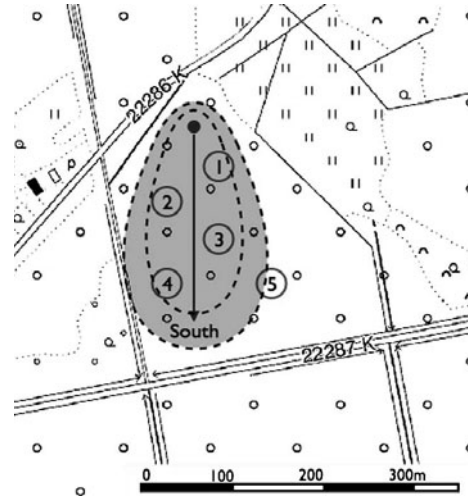

Fig. 1. Flux tower site at Järvselja, the blue dot marks the location of the tower and the numbered circles represent the five permanent sample plots. The broken lines denote the flux footprint in southern direction. The measurement cottage is located two meters in western direction at the base of the tower.

2. Materials and methods

2.1. Site description

The measurements were carried out at a site located in Järvselja Experimental Forest of Estonian University of Life Sciences, located in southeastern Estonia (58°25′N, 27°46′E). Järvselja is situated in the hemiboreal forest zone with a moderately cool and moist climate. Mean annual temperature is 4–6 °C. Annual precipitation is 500–750 mm, about 40–80 mm of this total is snow. The length of the growing season (daily air temperature above 5 °C) averages between 170 and 180 days. A scaffolding tower of 20 m height was used for the flux measurements above the forest canopy and the cumulative contribution (Horst and Weil, 1992) was 78% at a distance of 200 m upwind of the tower. The measurement cottage to house the sensors for the trace gas analysis and auxiliary equipment was established beside the base of the tower in 2008. For an overview of the location see Fig. 1. Both campaigns were covering the time of late summer and in 2008 the measurements started from 6th of August and ended at 27th August while 2009

Table 1
Overview on the measurement series conducted throughout both campaigns. In 2008 we conducted already several measurements, denoted by "preview", which have been used to set up the measurements in 2009.

	2008		2009	
	Time	Height over ground	Time	Height over ground
Flux measurements[a]	6th to 21st August	20 m and 2 m	12th to 19th August 19th to 25th August 25th Aug. to 9th September 9th to 21st September	20 m 2 m 20 m 2 m
Trace gases	6th to 21st August	20 m and 2 m	12th August to 21st September	20 m and 2 m
Particles	6th to 20th August	3 m	–	–
Leaf level BVOC and photosynthesis	11th to 17th August	16 m *B. pendula* 18–16 m *P. abies* 0–2 m understory Preview	24th to 27th August 2nd and 5th September	16 m *B. pendula* 0.5 m *T. cordata* 16–18 m *P. abies* 2 m *Q. robur* 1 m *B. pubescens* 1.5 m *P. tremula*
Soil fluxes CO_2 automatic chamber manual chamber	–14th to 15th August	– Preview	14th to 16th September 7th October/23rd November 7th to 11th September	0 m 0 m 0 m
Soil BVOC	–	–	25th, 27th to 29th August 7th to 8th September	0 m 0 m
Auxiliary[b]	6th to 21st August	20 m and 2 m	12th August to 21st September	20 m and 2 m

[a] Data of the year 2009 have been measured with one Eddy system on timely separated heights and were therefore not included into Fig. 1.
[b] Auxiliary measurements are air temperature, pressure, relative humidity, and global solar radiation.

the measurement campaign started at 12th August and ended at 23rd September. Table 1 gives an overview on the measurements that have been conducted during those times.

To characterize the forest ecosystem structure five circular permanent sample plots with 30 m diameter were established in the vicinity of the flux tower. These were located along a virtual line to the south at distances ranging from 50 to 200 m which distributes them such that they are located within the footprint area of the tower according to the main wind direction during that time of the year. Within those permanent sample plots all trees with a diameter at breast height (dbh) more than 6 cm were recorded to account for the tree number by species. Height measurements were conducted for 20% of trees per plot which have been randomly chosen. Each species has been handled as a separate element in that procedure. The age was determined by counting the rings on increment cores for one average tree per major species. The stand characteristics were calculated separately for the dominant (Table 2) and suppressed tree layers. The forest around the flux tower site is secondary growth managed with clear cuts of various sizes (1–5 ha) resulting in quite fine mosaic of stands. The regeneration of this type of stands is usually a combined method with planted coniferous and naturally established hardwoods in mixture. The young stands are treated to reduce the proportion of hardwoods in favor of the economically more valuable coniferous species (both planted and advance regeneration). This type of management which employs a natural regeneration after stand-replacing disturbances and following population control is the characteristic management according to medium intensity forestry (Metslaid et al., 2007) and a common practice in Estonian forestry. The plots were dominated by Norway spruce (*Picea abies* (L.) Karst.) and the forest site type according to the Estonian classification is the *Oxalis-Myrtillus* (Lõhmus, 2004) type. We found as co-dominant species silver birch (*Betula pendula* Roth.) and black alder (*Alnus glutinosa* L.) and due to the presence of other species such as *Populus tremula* L., *Alnus incana* (L.) Moench, *Tilia cordata* Mill., and *Fraxinus excelsior* L. the stand mixture is fairly complex. Of particularly importance is the presence of a suppressed tree layer (mean height of 6.4 ± 0.6 m over the five plots) which affects turbulent air flows in the stand. The main components of ground vegetation are *Oxalis acetosella* (L.), *Vaccinium myrtillus* (L.), *Calamagrostis arundinacea* (L.), *Convallaria majalis* (L.), and *Melampyrum pratense* (L.). The moss layer consists of several species and partly at humid spots we found *Sphagnum* spp. The mean N concentration in the aboveground litter was 1.53 ± 0.10%, and the mean C concentration 48.65 ± 0.72%.

As the site is located in the Lake Peipsi depression (Arold, 2005) and bordered by wetland massifs the soils of the site are strongly groundwater influenced, and employed a high groundwater table due to the vicinity to Lake Peipsi and the low altitudes of forest grounds. The soil around the flux tower showed a thick raw humus horizon with an average thickness of 24 cm, and the underlying sediment material was characterized by blue gleyic spots. The soil type was Haplic Gleysol (eutric) (WRB, 2006). Due to high clay content of the soil, the soil hydraulic conductivity is low; implying that the soils will stay wet longer times after snowmelt in spring. If affected by drought, even heavy rainfalls poorly penetrate into the soil and much of the precipitation is lost as runoff (Niinemets et al., 1999). To obtain data representing the area at larger scale, additional 4 sampling plots where established to the east direction of the flux tower. The soil types were Haplic Podsol and Haplic Stagnosol (WRB, 2006) and the forest site type *Oxalis-Myrtillus*.

2.2. Eddy covariance system

Eddy covariance (Baldocchi et al., 1988; Aubinet et al., 2000) CO_2, H_2O and energy flux measurements were conducted above the forest canopy on a 20 m tall tower (referred to as overstory flux measurements) and below the canopy at 1.5 m height above the ground (referred to as understory flux measurements). The three wind components and the speed of sound were measured by three-dimensional sonic anemometers (2008: CSAT3, Campbell Scientific, Logan, UT, USA; 2009: Metek USA-1, Metek GmbH, Elmshorn, Germany), CO_2 and H_2O mol densities/fractions in 2008 (over- and understory) by an open-path infrared gas analyzer (IRGA) (Li-7500) and in 2009 (over- and understory, but not at the same time) by a closed-path IRGA (Li-7000, both Li-Cor, Lincoln, NE, USA). In the closed-path system air was sucked from the inlet, mounted 0.1 m below the centre of the sensor volume of the sonic anemometer, through a 35 m Teflon tube of 0.008 m inner diameter through a filter (Acro 50, Gelman, Ann Arbor, MI, USA) to the closed-path IRGA deployed in a measurement cottage at the base of the tower at a flow rate of 15.2 L min^{-1} (KNF Laboport, KNF GmbH Freiburg, Germany). The closed-path IRGA was operated in the absolute mode, flushing the reference cell with dry N_2 from a gas cylinder at 0.1 L min^{-1}. The open-path IRGAs were mounted 0.2 m below the centre of the sensor volume of the sonic anemometer in a horizontal position. Sonic anemometers, the intake of the closed-path inlet tube, and the open-path IRGAs were oriented into the main wind direction to minimize flow distortion and longitudinal sensor separation to the sonic anemometers. In 2008, data from the sonic anemometers and the open-path IRGAs were acquired at 20 Hz and transferred via the SDM protocol to data loggers (CR3000, Campbell Scientific, Logan, UT, USA). In 2009, the data from the sonic anemometer was transferred by serial protocol with a frequency of 10 Hz to a computer and closed path IRGA data were transferred as analog signals with the same frequency and captured by a datalogger (Pico ADC20, Pico Technology, St. Neots, UK) connected to the same computer. Half-hourly average eddy fluxes were calculated from quality controlled flux data as the covariance between the turbulent departures from the mean of the vertical wind speed and the CO_2 and H_2O mixing ratios as described in detail in Haslwanter et al. (2009) and will thus not be repeated here. Net CO_2 and H_2O fluxes were calculated as the sum of the corrected vertical eddy term and the storage fluxes, the latter being estimated from the time-rate-of-change in scalar density at the respective reference height. Negative fluxes represent transport from the atmosphere towards the surface, positive ones the reverse.

2.3. Leaf level photosynthesis

We used a portable infrared gas analyzer (IRGA) cuvette system (Walz, GFS3000, Walz GmbH, Effeltrich, Germany) for leaf level photosynthesis measurements. Broad leaved species were measured using a clamping cuvette with parameter settings: flow rate of 1 L min^{-1}, 380 μmol mol^{-1} CO_2, 75–80% humidity and 25 °C. Conifers (*P. abies*) were measured using the conifer chamber (provided by Walz for GFS3000), where small twigs can be fitted in. To avoid clamping stress, twigs were selected and prepared 3 days before the measurements by removing a portion of the needles and wrapping the site of needle removal airtight in Teflon tape. Trees in the top part of the canopy (*P. abies* and *B. pendula*) were accessed from the scaffoldings located at the measurement site (2 towers of 16 and 20m height) and were measured at a height of 16 and 18 m (maximal canopy height about 20 m). To represent sun and shade conditions, we choose leaves that are exposed to south, west and north directions. Leaves of trees in the understory (*P. abies*, *T. cordata*, *B. pendula* and *B. pubescens*) were measured at a height between 0.1 and 1.5 m and are supposed to be all under shade condition during summer time where the canopy is densely closed by the leaves of the deciduous trees. Leaves of trees in forest gaps were measured at heights between 0.4 and 1.5 m and we choose mostly

Table 2
Description of the dominant canopy layer of sample plots at the atmosphere–forest measurement campaign site. The proportion of the tree species is calculated on volume basis of stand total wood volume.

Plot number	Stand volume (m^3 ha^{-1})	Number of trees (ha^{-1})	Proportion (%)					
			Picea abies	*Betula pendula*	*Alnus glutinosa*	Other	Age	Height
1	171.4	1789	46.6	46.0	0.6	7.4	28	14.3
2	72.8	2395	39.8	34.5	25.3	0.4	21	10.0
3	54.2	2621	46.7	41.1	12.0	0.2	29	8.9
4	116.7	2621	43.9	28.7	–	27.4	23	10.2
5	210.8	2015	57.6	39.6	1.7	1.1	27	14.4

sun exposed leaves to asses if there is difference to the sun exposed leaves in the top canopy.

2.4. Trace gas analyses

The trace gas analyzers have been installed within the measurement cottage. We took their inlet airstreams from above canopy by a Teflon tube that reached to the top of the tower and a bypass inlet at 2 m height to allow sampling also below canopy. The ozone was detected using a Thermo (Model 49*i*) ozone analyzer and $NO/NO_2/NO_X$ were detected with a Thermo (Model 42*i*), both Thermo Scientific, Waltham, MA, USA. During the campaign in 2008 we had additionally an air quality measurement transportable laboratory, equipped with instruments for NO_X (Horiba APNA-370), SO_2 (Horiba APSA-370) both from Horiba Jobin Yvon GmbH, Unterhaching, Germany and particulate matter (PM1, PM2.5 and PM10, Grimm EDM107, Grimm Aerosol Technik GmbH & Co. KG, Ainring, Germany) sensors, placed on the side of a small forest road 100 m west of the flux tower.

2.5. Manual soil chamber measurements of CH_4, N_2O and CO_2 soil fluxes

One plot was selected near to the understory eddy covariance system and two circular stainless steel collars with a diameter of 305 mm were inserted about 6 cm into the top soil. The placement of the collars took place in the beginning of the campaign in 2008 and measurements conducted refer to the campaign in 2009 (Table 1). The effective chamber volume (23.8 L) of the cylindrical stainless steel chambers was calculated from the average height from the soil surface to the top of the chamber. The chamber atmosphere was mixed by a small built-in fan to prevent the formation of gradients (Frenzel and Karofeld, 2000) and sealed air tight with water. Pre-installed thermocouple sensors connected to a thermocouple reader (Comark KM330) registered the temperatures in soil (−0.1 m, −0.15 m, −0.25 m of depth), inside the chambers, and in the atmospheric air (0 m, 0.5 m, 2 m of height).

Samples were drawn into 100 mL gas-tight syringes from the top of the chamber via a capillary (diameter 2 mm). Thirty seconds before each sampling, the capillary air was mixed for four times using the syringe. The samples were injected into 7 mL non-evacuated vials (Labco Exetainer 768W). The vials were flushed with 80 mL of sample air and over-pressurized with 20 mL of sample air. The chambers were operated in closed static mode and closed for a maximum of 30 min. 6–10 samples were collected during this time. These were stored in darkness at −18 °C and analyzed within 2 months after sampling by gas chromatography with a GC-FID/ECD (model HP 6890) and a GC-TCD (model HP 7890) as described by Syväsalo et al. (2004). All flux rates were calculated from the exponential change in gas concentrations in the chamber and temperature corrected (Appendix A). Air temperature inside the chambers was taken each time a sampling was conducted.

2.6. Automatic chamber measurements of CO_2 soil flux

Within the five circular sampling plots and at four reference locations outside of the sample plots, two stainless steel collars were placed in each, 7 m north and 7 m south from the plot center or at similar distance if outside a plot. The placement of the collars took place in the beginning of the measurement campaign 2009. In total, 18 collars of the size of 230 mm in diameter and 80 mm in height were installed and inserted about 2–3 cm into the top soil in order to eliminate diffusion through the soil away from the measurement area.

Soil respiration was measured using an automatic CO_2 Exchange System ACE (ADC BioScientific Limited, UK) equipped with a soil chamber, an infra-red gas analyzer (IRGA), a sensor for detecting photosynthetic active radiation, a soil moisture sensor (SM200, Delta-T Devices Ltd., Cambridge, England) and two temperature sensors. The soil chamber was equipped with a small fan for mixing the air. Volume of the lid of the soil chamber was 2.6 L. Upon measurement the lid was automatically placed tightly on the collar. Tight closing of the soil chamber was guaranteed by a rubber ring-seal in the contact surface between the rims of the lower part of the chamber (collar inserted under a special rim of the frame of the station) and its upper (guided) counterpart. The height of the collars from the soil surface was measured at every occasion. The ACE station was operated in closed mode with zero option. In this mode the increase of CO_2 in the chamber from soil activity is measured. The zero measurement is made by passing CO_2-free air through the chamber. In that mode, the chamber is operated as a closed static chamber (Pumpanen et al., 2004) and details about the data handling are given in Appendix A. Continuous measurements were conducted in one collar using the interval of 15 min between each measurement cycle of 5 min. Single repeated measurements were conducted upon the time of collar installments, during the main campaign, and later in October and November 2009. 2–10 sequential measurements were conducted. Simultaneously with the soil flux measurements, soil temperature and moisture were measured at the depth of 5 cm, and air temperature on the ground level.

Litter samples were collected in each plot. To distinguish the share of respiration originated from the fallen litter and from the soil, upon measurement the litter was removed in one collar (always the southern one) of each plot. The respiration from the decomposition of litter would result from the difference between the collars. The litter was taken to the lab, dry weight measured and analyzed for C and N contents.

2.7. BVOC sampling and analyses

We measured volatiles organic compounds (VOC) emitted from leaves in parallel with leaf physiological parameters by diverting a part of the Walz leaf and conifer cuvette outflow. The VOC's were adsorbed onto multibed stainless steel cartridges (10.5 cm length, 3 cm inner diameter, Supelco, Bellefonte, USA) filled with Carbotrap C 20/40 mesh (0.2 g), Carbopack C 40/60 mesh (0.1 g) and Carbotrap X 20/40 mesh (0.1 g) adsorbents (Supelco, Bellefonte, USA) at

a flow rate of 250 mL min^{-1} for 10 min. (altogether 2.5 L air). Background air samples were collected from the empty chamber before and after the measurements. Adsorbent cartridges were analyzed with a combined Shimadzu TD20 automated cartridge desorber and Shimadzu QP2010 plus GC-MS instrument (Shimadzu Corporation, Kyoto, Japan). The TD20 parameters, GC–MS conditions and compounds identification were presented in detail in Copolovici et al. (2009) and Toome et al. (2010).

From the manually operated soil chambers VOC samples were taken analogously as from the leaves using the same flow rates of 250 mL min^{-1} but sampling for 20 min. After sampling a background air sample, the chamber was closed for a period of 30 min and then the sample was taken onto the steel cartridge. To avoid underpressure in the water-sealed chamber, we allowed background air to enter via a small Teflon tube to the bottom of the chamber during sampling using a valve sealing that allows to open an inlet while sampling. That led to a change in the operation mode from a closed static chamber to an open dynamic chamber. The data handling is described in detail in Appendix A. Subsequent GC–MS analysis of the VOC soil emissions have been conducted as described above.

3. Results and discussion

3.1. Net ecosystem CO_2 exchange

The net ecosystem CO_2 exchange (NEE) above the canopy exhibited a clear diurnal cycle with net uptake during daytime and net loss of CO_2 during nighttime (Fig. 2 and Table 3). These fluxes fall in between those observed for European deciduous (Granier et al., 2003) and coniferous (Bernhofer et al., 2003; Ceulemans et al., 2003) forest ecosystems and likely reflect the mixed nature of the stand. In contrast, the NEE measured in the understory exhibited hardly any diurnal cycle and was positive for almost all of the time (Fig. 2 and Table 3). Consistent positive daytime understory NEE for summertime conditions were also reported by Misson et al. (2007) for 8 out of 11 study sites. The contribution of the storage flux to the NEE was negligible in the understory (Misson et al., 2007), while an appreciable negative and positive storage flux (Fig. 2) in the morning and evening, respectively, was detected with the overstory system indicative of venting and accumulation of canopy air, respectively (Aubinet et al., 2000). Flux divergence was observed during nighttime, when the respiration measured above the canopy was lower than in the understory (Fig. 2). This may reflect differences in the footprint between the over- and understory eddy covariance systems which generally differ greatly in extent (Baldocchi, 1997) and in addition sometimes differed in direction due to occasional decoupling of flow directions above and below the canopy (data not shown). The differences in nighttime over- and understory respiration may also be indicative of advection (Aubinet et al., 2000), part of the CO_2 being respired by the soil and understory vegetation potentially being advected (horizontally and/or vertically). In order to confirm the latter hypothesis, however, detailed measurements of within-canopy horizontal and vertical gradients in CO_2 concentration and wind speed would be required (Feigenwinter et al., 2008).

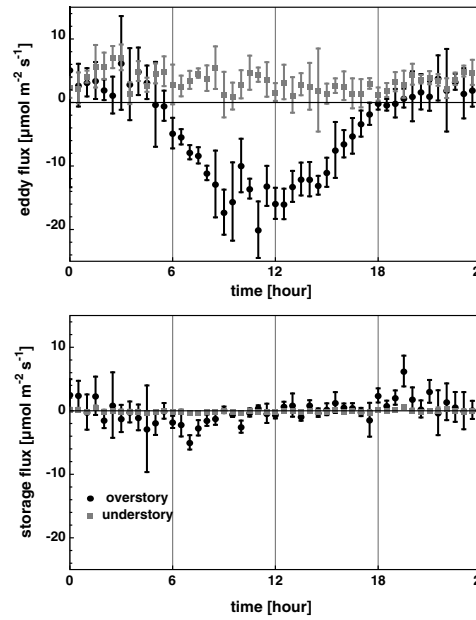

Fig. 2. Eddy fluxes (upper panel) and storage fluxes (lower panel). Overstory fluxes are black dots and understory fluxes gray squares. Median daily fluxes were compiled from the measurements in 2008 and error bars denote standard errors.

3.2. Reactive trace gases

3.2.1. Ozone and nitrogen oxides

The formation of tropospheric ozone is bound to the availability of sunlight and NO_X which provide the energy and the primary source of oxygen atoms, respectively. VOCs act as catalysts of this reaction (Atkinson, 2000; Atkinson and Arey, 2003). As seen in Fig. 3, ozone varied in a clear diurnal cycle above and below the canopy. Lowest values were found under nighttime conditions, while the highest values occurred during daytime. The peak in ozone concentrations above canopy was broader and somewhat skewed towards the afternoon, while below canopy ozone concentrations peaked around noon and reached nighttime levels about

Table 3
Mean values and standard errors of the ecosystem CO_2 fluxes (August 2008). All fluxes are expressed in µmol m^{-2} s^{-1}.

		Overstory			Understory		
			Min	Max		Min	Max
Daily total	f_{cn}	−4.59 ± 1.01	−19.83	4.56	3.60 ± 0.23	1.19	7.15
	f_{sn}	0.1 ± 0.3	−4.97	6.26	0.03 ± 0.03	−0.35	0.66
Daytime	f_{cn}	−10.28 ± 0.99			2.93 ± 0.99		
	f_{sn}	−0.5 ± 0.3			−0.04 ± 0.03		
Nighttime	f_{cn}	2.92 ± 0.25			4.34 ± 0.34		
	f_{sn}	0.74 ± 0.41			0.09 ± 0.04		

f_{cn}, net CO_2 flux; f_{sn}, net storage flux.

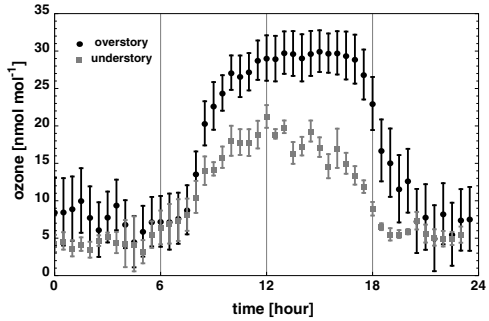

Fig. 3. Comparison between the ozone mixing ratios above and below the canopy. The data sets have been combined from both measurement campaigns. Ozone data was acquired with 1 s intervals, and half hourly averaged. From the combined data sets, medians have been calculated and error bars denote standard errors.

1 h earlier. Typical daily variations in ozone concentration above forest canopies have been reported to span the range from 10 to 70 nmol mol^{-1} (Mikkelsen et al., 2000), which is well represented by our findings as well.

NO and NO_2 concentrations showed as well diurnal cycles where NO ranged between 0.05 and 0.3 nmol mol^{-1} and NO_2 between 0.2 and 1.5 nmol mol^{-1} with lower values during nighttime. A slight canopy gradient with smaller values below was found. Nitrogen oxides, as originated from burning processes and soil efflux, were found to correlate with wind direction (Fig. 4). Westerly winds caused higher NO and NO_2 concentrations, while smallest concentrations were associated with easterly winds. Cities and settlements as well as an access road to the region are located to the West of the measurement station. Ozone concentrations followed that same pattern and were highest on days with West to South-West winds and lowest with winds from the East (Fig. 4).

Ozone uptake to forest canopies has been reported for temperate and boreal ecosystems (Mikkelsen et al., 2004; Rannik et al., 2009). That uptake is mostly reported to occur through stomata, surface deposition and also by reaction with emitted BVOC. While the difference in the ozone concentrations above and below the canopy are suggesting that there should be a net ozone uptake by the canopy the question remains to what extend the different environmental conditions above and below the canopy influence that flux.

3.2.2. Particulate matter and anthropogenic influences

In order to assess possible anthropogenic influences on the location of the flux tower we compared the measurements of the air quality measurement bus and the measurements within and above the canopy. Table 4 summarizes the results. If compared to the reported Estonian yearly background values (Pajuste et al., 2004), the situation found in Järvselja is about 2–3 times lower. Sulfur dioxide (SO_2) is predominantly of anthropogenic origin and plays a role in acid rain formation, which has a negative impact on forest ecosystems and tree growth. The values found indicate, that the measurement site is not much influenced by SO_2 emissions. The same picture was found for NO_X. Especially the low NO values, direct tracers of nearby combustion, support the conclusion that Järvselja is located in an area with low human impact on air pollution.

Particulate matter concentrations (PM1, PM2.5 and PM10) also remained far below the national threshold (50 μg m^{-3}, averaged over 24 h for a period of 20 years) and characterize the site as between "remote" and "non-urban continental" (Seinfeld and Pandis, 2006). In conclusion, the measurement site at the flux tower in Järvselja can be graded as an atmospherically clean and low anthropogenic impacted site.

3.2.3. BVOC emissions

To assess the state of the atmosphere and the flux of carbon within the forest ecosystem at Järvselja, we also conducted measurements of leaf level and soil BVOC fluxes. These were

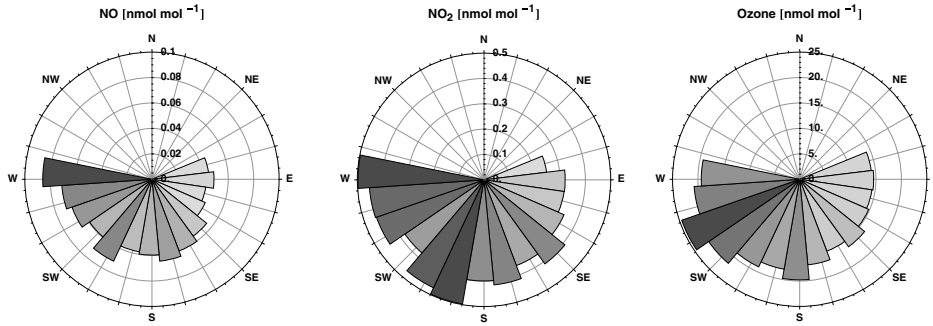

Fig. 4. Relationship between the wind direction and the mixing ratios of nitrogen oxides (NO and NO_2) and ozone during the measurement campaign in 2009. Darker color refers to higher mixing ratios.

Table 4
Comparison of trace gases and particles (μg m^{-3}) measured at the roadside (bus) and the flux tower in the forest (cottage).

	NO	NO_2	SO_2	O3	PM1	PM2.5	PM10
Location	Bus	Cottage	Bus	Cottage	Bus		
Instrument	APNA 370	TEI 42*i*	ASNA 370	TEI 49*i*	Grimm monitor N 180		
Median	0.1–0.7	0.2–0.9	0	43.33	4	6	14.50
95%	1.2–1.5	1.2–2.3	0.4	78.2	12	20.0	75.0
5%	0–0.2	0–0.2	0	5.71	2	2.90	5.50

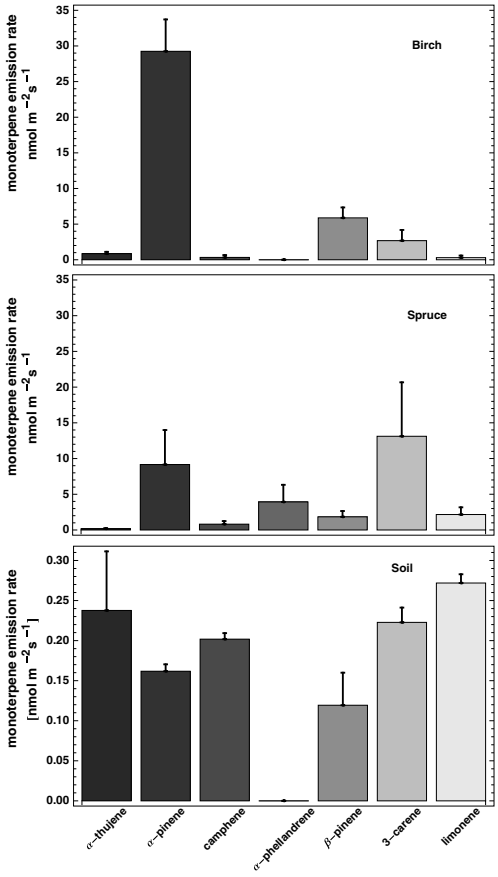

Fig. 5. Monoterpene emissions from the main tree species *B. pendula* and *P. abies*, averaged over leaves and branches differently exposed to sunlight. We used in both cases West, South and North exposed leaves or branches. The lower panel shows monoterpene emissions from soil and leaf litter. Error bars indicate standard errors.

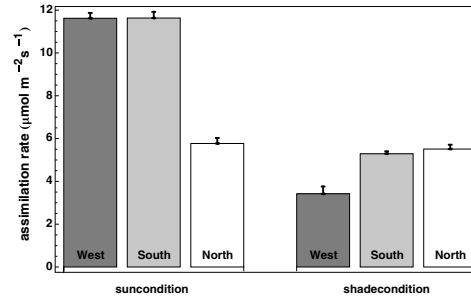

Fig. 6. Photosynthetic net assimilation rate of *B. pendula* on leaf level, averaged over 3–5 leaves. To simulate high light conditions (denoted sun condition) the measurement was conducted with a quantum flux of 1000 μmol m^{-2} s^{-1} and shade conditions were simulated by a quantum flux of 200 μmol m^{-2} s^{-1}. Directions given in the bars refer to the direction in which the leaves measured have been exposed. Error bars denote standard errors.

focused on isoprene and monoterpenes. Largest monoterpene emission rates (sum of monoterpenes, 46 ± 9 nmol m^{-2} s^{-1}) were found from *Betula* with α-pinene as the major monoterpene emitted. *P. abies* emitted a total monoterpene flux of 33 ± 11 nmol m^{-2} s^{-1}, where Δ^3-carene was the main contributing monoterpene followed by α-pinene and α-phellandrene (Fig. 5). The isoprene fluxes for *B. pendula* (1.2 ± 0.6 nmol m^{-2} s^{-1}) and *P. abies* (1.02 ± 0.36 nmol m^{-2} s^{-1}) showed no substantial differences. Compared to the total monoterpene emissions, isoprene emissions were about 45 times smaller.

Total soil monoterpene fluxes were found to be 40 times smaller (1.22 ± 0.16 nmol m^{-2} s^{-1}) than leaf emission rates. As isoprene is synthesized from recently fixed carbon by plants, the very small flux from the soil (0.05 ± 0.01 nmol m^{-2} s^{-1}) may be caused by the enclosed small plants and mosses which are known to emit isoprene as well (Tiiva et al., 2009). Another possibility is that soil bacteria are also involved in the isoprene fluxes – release and consumption of isoprene by soil bacteria has been reported (Cleveland and Yavitt, 1998; Scholler et al., 2002). The composition of monoterpene soil fluxes (Fig. 5) shows some interesting patterns as compared to the monoterpene emissions from the leaves of the species mainly contributing to the leaf litter in the soil O-horizon. We found, that α-phellandrene is lacking completely and that the main contributing monoterpenes at the leaf level, α-pinene and Δ^3-carene, are less abundant in the soil monoterpene fluxes. Instead, α-thujene and limonene, which have been found less abundant at the leaf level, are main contributors to the soil monoterpene fluxes. It is known, that monoterpene emission patterns are changing with the seasons and that stored monoterpenes, i.e. from resin ducts, exhibit different patterns than emission from newly synthesized monoterpenes (Hakola et al., 2003; Vuorinen et al., 2005; Holzke et al., 2006; Räisänen et al., 2008). Our result suggest, that α-thujene and limonene may be stored to greater amounts in the leaf tissues related to their synthesis when still attached to the branches and that α-phellandrene seems not to be stored in substantial amounts. Given the fact, that deciduous leaves mostly do not build specialized storage organs we can speculate that soil monoterpene emissions are caused by the coniferous litter. The contribution of the different litter parts, needles, leaves, cones etc. to the terpene fluxes remains to be investigated for our measurement site. As it is known that beside the leaf litter soil fungi and roots also emit different volatiles (Leff and Fierer, 2008) their contribution to the total emitted soil fluxes presented here have to be assessed in future.

3.3. Leaf level photosynthesis

Betula pendula is the most abundant, deciduous tree species present in both over- and understory of the experimental site. Leaf level net photosynthetic rates showed a clear dependency on and adaptation to light availability. *Betula* leaves at the top of the canopy (about 16–18 m) showed highest net assimilation rates when exposed to light fluxes of 1000 μmol quanta m^{-2} s^{-1}. Leaves that exposed to south and west directions were found to exhibit twice as high net assimilation (11.6 ± 0.3 μmol m^{-2} s^{-1}) as compared to north exposed leaves (5.7 ± 0.4 μmol m^{-2} s^{-1}; Fig. 6). To simulate also shade conditions, the same leaves were measured with a light flux of 200 μmol quanta m^{-2} s^{-1}. In this case, lowest net assimilation rates were measured in west exposed (3.5 ± 0.6 μmol m^{-2} s^{-1}) and highest in north exposed (5.5 ± 0.3 μmol m^{-2} s^{-1}) leaves (Fig. 6). Photosynthetic photon flux density (PPFD) in the top of the canopy was between 130 and 1230 μmol quanta m^{-2} s^{-1} for south- and west-exposed leaves

Table 5
CO_2 assimilation and transpiration from tree leaves in the understory at a photon flux density of 200 μmol quanta m^{-2} s^{-1}. Values shown are mean ± standard error of 3 different trees (3–7 measurements per tree).

Species	CO_2 assimilation (μmol m^{-2} s^{-1})	Transpiration (mmol m^{-2} s^{-1})	*N*
Betula pendula[a]	2.18 ± 0.13	0.78 ± 0.10	7
Betula pubescens	1.97 ± 0.14	1.03 ± 0.10	5
Tilia cordata	3.79 ± 0.27	1.12 ± 0.10	3

[a] Overstory assimilation values measured under 200 μmol quanta m^{-2} s^{-1} (shade condition) are inserted from data given in Fig. 5 for comparison.

and between 261 and 77 μmol quanta m^{-2} s^{-1} for north-exposed leaves. Interestingly, the photosynthesis of north-exposed *Betula* leaves in the top of the canopy seemed to be light-saturated already at a rather low photon flux density of 200 μmol quanta m^{-2} s^{-1}. Increasing light to 1000 μmol quanta m^{-2} s^{-1} increased photosynthesis only marginally, while the photosynthesis rate of west- and south-exposed leaves was about doubled (Lichtenthaler et al., 2007). Adaptations of the photosynthetic apparatus to light intensities are explaining that behavior (Niinemets et al., 1998, 1999, 2004).

P. abies is the most abundant tree species in the overstory of the measuring site. Net assimilation rates measured at 16–18 m height and a light flux of 1000 μmol quanta m^{-2} s^{-1} were 7.9 ± 1 μmol m^{-2} s^{-1}. Although *P. abies* net assimilation rate is lower than that of *B. pendula* (as was to be expected), it is still appreciable and in the normal to high range (Wallin et al., 1990; Marek et al., 1997). The expositions of the branches measured have been West, South and North.

Net assimilation rates of *B. pendula* leaves in the understory (apparent light was between 6 and 69 μmol quanta m^{-2} s^{-1}) were about 2 μmol m^{-2} s^{-1} when measured with a light flux of 200 μmol quanta m^{-2} s^{-1} (Table 5) and therefore much lower than overstory leaves measured under the same conditions (Fig. 6). For *B. pendula*, a light-requiring species, only small seedlings were found in the understory. Another typical pattern in forest ecosystems are gaps due to fallen or removed trees. We measured also in such gap situations. Light conditions there were much improved compared with the understory (apparent light was between 7 and 824 μmol quanta m^{-2} s^{-1}) and due to that we measured again with 1000 μmol quanta m^{-2} s^{-1}. The net assimilation rate of *Betula* leaves were very variable, ranging between 5.6 and 9.6 μmol m^{-2} s^{-1}, at average about 8 μmol m^{-2} s^{-1}, almost as high as in the overstory (Table 6). Of the species measured in the understory (3 species shown in Table 6), *Tilia* had higher net assimilation rates than the two *Betula* species. The gap offered a niche for those species, which were not found in the understory, like *Quercus robur* and *Populus tremula*. In general, the canopy discriminates the irradiative energy input strongly. In particular, the photosynthetic active radiation (PAR) input that is absorbed more strongly than near infra-red light can be even less than 1–2% of above-canopy value in these types of forests (Niinemets et al., 1998).

Table 6
CO_2 assimilation and transpiration from tree leaves in a forest gap at a photon flux density of 1000 μmol quanta m^{-2} s^{-1}. Values shown are mean ± standard error of 3 different trees (4–8 measurements per tree).

Species	CO_2 assimilation (μmol m^{-2} s^{-1})	Transpiration (mmol m^{-2} s^{-1})	*N*
Quercus robur	5.14 ± 1.22	1.46 ± 0.32	4
Populus tremula	8.24 ± 0.88	2.97 ± 0.09	5
Betula pendula	8.03 ± 1.23	2.29 ± 0.25	8

Table 7
Mean soil CO_2 effluxes and key environmental variables at Järvselja, measured with the automatic chamber system during the campaign in 2009. Time of measuring was between 12 and 17 h. Data given as mean (of 2–10 measurements) ± standard error.

Date	16 September	7 October	23 November
Soil efflux, μmol CO_2 m^{-2} s^{-1}	8.40 ± 0.82	9.60 ± 0.04	5.45 ± 0.53
T_{air}, °C	13.3 ± 0.3	8.4 ± 0.0	6.3 ± 0.1
T_{soil}, °C	12.6 ± 0.1	8.4 ± 0.1	5.7 ± 0.0
PPFD, μmol m^{-2} s^{-1}	5.30 ± 1.14	21.50 ± 1.50	5.00 ± 1.10
Soil moisture % (v/v)	67.0 ± 5.8	69.1 ± 0.5	81.9 ± 10.9
Number of collars used	3	1	3

3.4. Soil chambers

3.4.1. Soil CO_2 efflux

Manual chamber measurements resulted in a mean soil CO_2 efflux of 3.54 ± 0.62 μmol m^{-2} s^{-1}. Compared to boreal soil fluxes ranging between 2 and 6 μmol m^{-2} s^{-1} in summer (Rayment and Jarvis, 2000; Kolari et al., 2006, 2009) and cool temperate forest ecosystem soil fluxes ranging between 2 and 8 μmol m^{-2} s^{-1} (Lee et al., 2002; Yuste et al., 2005) depending on the species contribution the values found in Järvselja fit into that picture. The average air temperature recorded on the soil surface was 17.7 ± 1.3 °C and the average soil temperature at −0.1 m was 13.6 ± 0.5 °C. Mean soil CO_2 efflux was 4.29 ± 0.11 μmol m^{-2} s^{-1} over the continuous measurement period of 72 h with the automatic chamber system. Average volumetric soil moisture was 51.36% (max 56.97%, min 45.71%), the average air temperature 10.2 ± 0.2 °C, and the average soil temperature 11.7 ± 0.1 °C during the days of measurements. The average daytime temperature from 9 to 18 h was very stable at 11.9 ± 0.3 °C. Results of the additional single measurements are presented in Table 7. In both cases, manual and automatic chamber measurements, we found rather stable emissions over the course of the day which is in accordance with the stable soil temperature during the measurements. As the chambers have been operated under shade conditions in the understory, the average temperature change during measurements have been 0.9 ± 0.5 °C. The data, obtained on both plots, give hints about the plasticity which occurs through changes in soil type and canopy structure and may be reflected by substantial changes in soil CO_2 efflux.

Even though soil respiration is known to correlate positive with soil temperature which is influenced by soil moisture and PAR (Lloyd and Taylor, 1994; Ruehr and Buchmann, 2010), we found only weak correlations which is mainly caused by the limited number of the measurements during the campaign in 2009.

Comparison of the two methods of continuous measurement cycles (Fig. 7) revealed that greater values of CO_2 efflux were recorded with the automatic chamber system. Even though, the mean daytime air temperature was by 5.8 °C lower and there was one week difference between the first conducted manual chamber measurements. Given the temperature dependency of soil respiration, this is a contradictory result, which may be reconciled based on differences in the sampling and analysis techniques employed. Among the technical explanations, site heterogeneity, different depth or the time of collar installation might have played a role as well.

Forest floor soil respiration measured may represent more than 75% of total ecosystem respiration (Solondz et al., 2008). Compared with the results of the understory eddy covariance fluxes as given by Table 3 our chamber fluxes exceeded the daytime eddy fluxes as measured in 2 m height by about 20% for the manual chamber measurements and on the whole day basis about 19% as com-

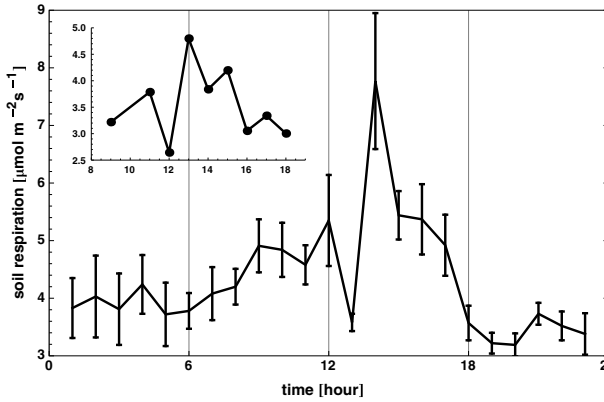

Fig. 7. Daily mean soil respiration measured over 3 days during the 2009 campaign by the automatic chamber system. Error bars denote standard errors of hourly measurements ($N = 2$–9). The inset shows a one day data set of soil respiration fluxes measured with the manual chamber system.

pared to the mean fluxes from the automatic chamber over the 24 h period. Given the differences in footprint between both methods (Baldocchi, 1997), the random and systematic uncertainties associated with each method (Hollinger and Richardson, 2005), and finally the differing time frames during which these measurements were made, we consider these results as very encouraging.

3.4.2. CH_4 and N_2O soil fluxes

Fluxes of methane and nitrous oxide were measured using the manual chamber system. We found negative methane fluxes during the campaign in 2009, which indicates a possible net consumption of CH_4 in soil (Chan and Parkin, 2001; Mori et al., 2008) which was also reported for boreal forest ecosystems (Whalen et al., 1992) or a possible atmospheric reaction of methane with hydroxyl radicals given sufficient high levels of NO_X (Wuebbles and Hayhoe, 2002) enclosed into the chamber. It is further known, that dry soils are sinks and wet soils act as sources for atmospheric methane as well as that tree species affect the source and sink properties of forest soils (Saari et al., 1998; Borken and Beese, 2006). The daytime average CH_4 flux was -0.69 ± 0.36 nmol m^{-2} s^{-1}. If compared to boreal soil methane fluxes in Hyytiälä, Finland (61°84′N, 24°29′E) of about -1.4 nmol m^{-2} s^{-1} (Pihlatie et al., 2009a) we found a slightly lower sink flux. The inverse relationship between the CO_2 and CH_4 fluxes may support the idea of methane consumption by methanotrophic bacteria in the soil as a reason for the flux.

We found daytime nitrous oxide emissions that averaged 0.18 ± 0.09 nmol m^{-2} s^{-1} which are about the same compared to soil N_2O fluxes at Hyytiälä of 0.15 nmol m^{-2} s^{-1} (Pihlatie et al., 2009b). N_2O is mainly produced by denitrification process in soils. In deeper layers, given wet and anaerobic conditions denitrification may occur frequently (Ball et al., 1999; Yamulki and Jarvis, 2002). In our groundwater influenced study site, the soil temperature should positively correlate with the N_2O efflux (Schindlbacher et al., 2004). Due to the limited number of measurements we observed only a weak positive correlation.

4. Conclusions and outlook

One might argue, and rightfully so, that the two measurement campaigns conducted at the Järvselja Experimental Forest station during the summers 2008 and 2009 only allowed us to take a snapshot of the multitude of trace gas fluxes which occur on a continuous basis between this forest and the atmosphere. While this is likely to be correct, we strongly believe that the two measurement campaigns already unearthed a number of encouraging results which may serve as building blocks upon which we aim to continue research at this site. We have characterized the structure and composition of the forest and the soil, we conducted above- and below-canopy eddy covariance CO_2 and H_2O flux measurements, quantified leaf photosynthesis, transpiration and isoprene/monoterpene emissions, soil CO_2, methane, nitrous oxide and monoterpene fluxes, as well as the above- and within-canopy concentrations of several reactive trace gases and particulate matter. Our major findings are that (i) in terms of the magnitude of the CO_2 exchange this mixed forest ranged in between boreal coniferous and temperate broad-leaved forests, that (ii) understory eddy covariance CO_2 fluxes and manual/automated soil respiration rates agreed reasonably, (iii) leaf and soil monoterpene emissions had contrasting signatures, (iv) soils were sinks for methane and sources for nitrous oxides, and (v) from an air quality point of view the study site was characterized as remote/rural. Future experimental efforts will be directed towards year-round flux measurements of a larger number of trace gases, of which some sources/sinks, such as of methane, nitrous oxide, isoprene and monoterpenes, were already characterized at the leaf and soil level. In a next step, these measurements both at the ecosystem and leaf/soil scale should be assimilated into process-oriented models of forest–atmosphere exchange in order to test our quantitative ability of simulating these processes. Combining these two lines of information, experimental field data and simulation analysis, will be critical in order to finally assess the role this and similar forests in this region are playing in modulating climate and how sensitive these ecosystems are to likely future climate.

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Appendix A.

We describe the concentration change in time by the flux from soil into a chamber by an exponential relation (Pedersen et al., 2001; Pumpanen et al., 2001, 2004):

$$C(t) = C_0 e^{kt} \quad (1)$$

where C_0 denotes the background concentration that is enclosed inside the chamber at the beginning of the closing period. In case of the manual chamber, the sampling procedure leaded to a series of data points $d = \{t_0, C_0, \ldots, t_n, C_n\}$, which have been used to estimate the flux rate k by non-linear least-square fit of Eq. (1) to d. To calculate the molar flow F_c, we apply:

$$F_c = C(t_n)\frac{V}{A} \cdot \frac{1}{V_m} \quad (2)$$

with a given chamber volume V in m^3, the covered area A in m^2, and the molar volume V_m of 22.4 L mol^{-1}. We further assumed that the pressure stayed constant throughout the sampling period and corrected for the temperature changes by multiplying Eq. (2) with:

$$\xi(T) = \frac{T_0 - T}{T_0} \quad (3)$$

where T_0 is 273.15 K and T the temperature as measured from the chamber sensor in Kelvin.

We conducted also the BVOC sampling by usage of the manual chamber system. In this case, it is only possible to apply a data set of two measurement points $d = \{t_0, C_0; t_1, C_1\}$, with the sample at t_0 given the background concentration and at t_1 the sample which is sucked onto the Carbotrap cartridge. Rearranging equation 1 and application of the logarithm leaded to:

$$\log(C(t_1) - C(t_0)) = k \quad (4)$$

and we applied again Eqs. (1)–(3) to achieve the temperature corrected molar flow of BVOC emitted from the top soil layer including leaf litter at the start of the BVOC sampling procedure. While the amounts of aliquot we took from the manual chambers volume (23 L) with the syringes are neglectable if compared to the total chamber volume that is not true in case of the BVOC measurements where we took an aliquot of five liters per sample over a period of 20 min. To prevent underpressure we let stream in background air to the bottom of the chamber with a small tube while sampling and by that changing instantaneously the concentration within the chamber. That resembles the open flow chamber system as described by Pumpanen et al. (2001) and can be described by a budget function assumed that there were no leak flows and the sample was taken with a flow q_1:

$$\frac{dC_i}{dt} = Q + q_1(C_0 - C_i) \quad (5)$$

where Q denoted the flow from the soil into the chamber while sampling and C_0, the background air replacing the air drawn into the cartridge. Integration of Eq. (5) and rearranging with respect to Q leads to a timely description of the flux added while sampling:

$$Q(t) = q_1(-C_0 + C_i - e^{-q_1 t}) \quad (6)$$

With that, we could account for the portion of soil efflux that have been generated during the 20 min sampling period and have been added to chamber.

The automatic chamber system measured the fluxes directly relative to a CO_2 free air standard and resembles in its operation therefore also a closed static system (Pumpanen et al., 2004). The software of the automatic chamber system used the method as given by Pedersen et al. (2001) to calculate the chamber flows. Measurements that did not reach a stable fluxes have been discarded by the system.

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Synthesizing greenhouse gas fluxes across nine European peatlands and shrublands – responses to climatic and environmental changes

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Abstract. In this study, we compare annual fluxes of methane (CH_4), nitrous oxide (N_2O) and soil respiratory carbon dioxide (CO_2) measured at nine European peatlands ($n = 4$) and shrublands ($n = 5$). The sites range from northern Sweden to Spain, covering a span in mean annual air temperature from 0 to 16 °C, and in annual precipitation from 300 to 1300 mm yr^{-1}. The effects of climate change, including temperature increase and prolonged drought, were tested at five shrubland sites. At one peatland site, the long-term (> 30 yr) effect of drainage was assessed, while increased nitrogen deposition was investigated at three peatland sites.

The shrublands were generally sinks for atmospheric CH_4, whereas the peatlands were CH_4 sources, with fluxes ranging from −519 to +6890 mg CH_4-C m^{-2} yr^{-1} across the studied ecosystems. At the peatland sites, annual CH_4 emission increased with mean annual air temperature, while a negative relationship was found between net CH_4 uptake and the soil carbon stock at the shrubland sites. Annual N_2O fluxes were generally small ranging from −14 to 42 mg N_2O-N m^{-2} yr^{-1}. Highest N_2O emission occurred at the sites that had highest nitrate (NO_3^-) concentration in the soil water. Furthermore, experimentally increased NO_3^- deposition led to increased N_2O efflux, whereas prolonged drought and long-term drainage reduced the N_2O efflux. Soil CO_2 emissions in control plots ranged from 310 to 732 g CO_2-C m^{-2} yr^{-1}. Drought and long-term drainage

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generally reduced the soil CO_2 efflux, except at a hydric shrubland where drought tended to increase soil respiration.

In terms of fractional importance of each greenhouse gas to the total numerical global warming response, the change in CO_2 efflux dominated the response in all treatments (ranging 71–96 %), except for NO_3^- addition where 89 % was due to change in CH_4 emissions. Thus, in European peatlands and shrublands the effect on global warming induced by the investigated anthropogenic disturbances will be dominated by variations in soil CO_2 fluxes.

1 Introduction

Shrublands are natural or semi-natural nutrient poor ecosystems, characterized by patches of low stature vascular vegetation alternating with bryophytes or bare soil. Commonly, the shrublands on poorly drained, deep organic soils (> 30 cm) are called peatlands (Lai, 2009) while shrublands on mineral soils, which are typically excessively drained, are called shrublands sensu stricto (Beier et al., 2009). These ecosystem types make up approximately 7 % of the European land area (EEA, 2006; Montanarella et al., 2006) and may contribute valued ecosystem services, such as biodiversity, habitat provision, recreation, water purification and carbon (C) sequestration (Wessel et al., 2004; Kimmel and Mander, 2010). For instance, northern peatlands were estimated to sequester 0.07 Gt C yr^{-1}, which over time has accumulated, resulting in deep organic soil profiles that constitute a major store of terrestrial C (Gorham, 1991; Clymo et al., 1998). Peatlands and shrublands are sensitive to disturbances such as climate change and management, and the responses to these anthropogenic disturbances are therefore important for the overall European greenhouse gas (GHG) budget and other ecosystem services.

In Europe, peatlands are most abundant under the subarctic and temperate climates of the Nordic countries, where Finland and Sweden together account for 65 % of the European area of peat soils (Montanarella et al., 2006). The geographical distribution of shrublands in Europe includes the heather-moorlands on the British Isles, dry *Calluna* heaths along the Atlantic coastlines from northern Norway to northern Portugal, and finally maquis ecosystems under the Mediterranean climate in southern Europe (Beier et al., 2009).

Peatlands and shrublands share some common properties concerning vegetation and nutrient status, but they also differ fundamentally, especially in terms of hydrology, with related differences in soil structure, thermal properties and redox state. Such differences may lead to deviation in their respective drivers of GHG fluxes. Methane (CH_4) fluxes between soil and atmosphere represent the balance between CH_4 production and CH_4 oxidation. Peatlands are generally net CH_4 sources, where CH_4 efflux is controlled by water table depth, temperature and availability and quality of carbon substrates (Christensen et al., 2003; Lai, 2009). Presence and composition of vascular vegetation can also affect the CH_4 flux dynamics, both through root exudation of labile C substrates for CH_4 production (Ström et al., 2003), and by providing gas conduits, which may influence CH_4 production, oxidation and transport processes (Joabsson et al., 1999). In contrast, dry *Calluna* heaths and Mediterranean shrublands are usually CH_4 sinks, where CH_4 uptake most often correlates positively with temperature and negatively with soil water content (Castaldi and Fierro, 2005; Carter et al., 2011). The latter is due to the slower gas diffusion in water than in air, which causes soil water to limit CH_4 diffusion towards the zone of methanotrophic activity (Dunfield, 2007), mainly located in the upper 20 cm soil layer (e.g. Roslev et al., 1997).

Peatlands and shrublands also differ in the key drivers of nitrous oxide (N_2O) fluxes, which may involve at least two microbial processes, nitrification and denitrification, occurring under aerobic and anaerobic soil conditions, respectively (Baggs, 2011). Generally, the rates of these processes increase with nitrogen (N) availability. In a shallow peatland in Scotland, a spatial analysis revealed a negative correlation between N_2O emissions and soil pH, possibly an indirect result of limited soil N availability for the microbes at locations with higher pH values (Dinsmore et al., 2009), where the vegetation constitutes a stronger N sink. The temporal N_2O dynamics were primarily controlled by soil temperature with an apparent switch from consumption to production at about 8 °C (Dinsmore et al., 2009). Fluxes of N_2O in shrublands have only been investigated in a few studies, and knowledge of key drivers specific for this ecosystem type is sparse (e.g. Curtis et al., 2006; Carter et al., 2011). In four British moorlands, Curtis et al. (2006) detected either very low or no N_2O emission. Incubation of soil cores, however, demonstrated that N_2O efflux could be induced by warming and N addition. In a Danish *Calluna* heath, both soil moisture and temperature stimulated N_2O emissions (Carter et al., 2011).

Soil respiratory CO_2 emission originates from decomposition of soil organic matter and from plant root respiration. Together with gross ecosystem photosynthesis, soil respiratory CO_2 emission is the dominant flux of carbon between terrestrial ecosystems and the atmosphere (Schlesinger and Andrews, 2000), and changes in soil respiration in response to climate treatments is an important indicator for ecosystems' feedback to climate change. Soil CO_2 effluxes from peatlands typically correlate positively with both temperature and water table depth (Smith et al., 2003; Danevčič et al., 2010). Similarly for shrublands, soil CO_2 emissions were shown to be strongly controlled by temperature in an analysis based on two years of data from four European heathlands (Emmett et al., 2004).

The relative contribution to global warming of the three greenhouse gases CH_4, N_2O and soil respiratory CO_2 may vary between peatlands and shrublands. For instance, annual

GHG budgets for a minerotrophic fen in Finland and a shallow peatland in Scotland showed that N_2O fluxes were generally of minor importance, whereas CH_4 emissions and net ecosystem CO_2 exchange exerted the greatest impact on the ecosystem's contribution to global warming (Drewer et al., 2010). For comparison, in an old *Kunzea ericoides* shrubland in New Zealand, GHG emissions were dominated by CO_2 release from the soil while pronounced CH_4 uptake more than counteracted N_2O emissions on a CO_2-equivalent basis (Price et al., 2010). Similar studies for European shrublands are currently lacking.

In future, the exchange of GHGs between ecosystems and the atmosphere may be affected by climate change, including increasing temperatures and increased duration and frequency of summer droughts; the latter expected in western and southern Europe (IPCC, 2007a). For instance, Emmett et al. (2004) reported 0–19 % increases in soil CO_2 effluxes in response to 0.5–2 °C warming across four of the five shrublands described in this study. Furthermore, Carter et al. (2011) found a 20 % increase in CH_4 uptake rates under elevated temperature in a dry temperate heathland (DK-Bra in this study). Emissions of N_2O from this site were reduced and stimulated by drought and elevated temperature, respectively, but only when these treatments were combined with elevated atmospheric CO_2 concentrations (Carter et al., 2011).

In addition to climate change, the hydrology of peatlands is affected by other anthropogenic disturbances, such as extensive drainage operations to enable forestry, agriculture or peat harvesting. Drainage of peatland was particularly widespread in the northern temperate zone before the 1980s (Kimmel and Mander, 2010), leading to elevated CO_2 effluxes (von Arnold et al., 2005) and to decreased CH_4 emissions (Roulet and Moore, 1995), although emissions of CH_4 can be significant from drainage ditches in nutrient-rich fens (Minkkinen and Laine, 2006). By contrast, N_2O emissions from ombrotrophic bogs are relatively insensitive to changes in the water table, as the efflux is probably limited mainly by slow N transformation rates caused by low pH and nutrient availability (Martikainen et al., 1993).

A less well understood anthropogenic influence on GHG fluxes in peatlands and shrublands is the effect of enhanced atmospheric N deposition, originating from intensive agriculture (reduced N) and fossil fuel combustion (oxidized N). The global N pollution is unlikely to decline in the immediate future (Galloway et al., 2004; Peñuelas et al., 2012). Bragazza et al. (2006) investigated peatlands along an atmospheric N deposition gradient ranging from 2 to 20 $kg\,N\,ha^{-1}\,yr^{-1}$ and found increased soil respiratory CO_2 emissions under higher N deposition rates. This relationship was explained by the removal of N constraints on microbial metabolism and by improved litter quality. The effects of increased N availability on CH_4 exchange are dependent on site specific properties. Some soils show inhibitory effects on CH_4 oxidation (Crill et al., 1994; Christensen et al., 1999), while others show minor or no effects (Saarnio and Silvola, 1999). If the abundance of vascular plants increases in peatlands as a result of higher N availability, this may increase CH_4 emissions by providing gas conduits and improving litter quality (Joabsson et al., 1999; Nykänen et al., 2002). Increasing the amount of inorganic N in a soil will also enhance the potential for N_2O emissions both through nitrification and denitrification processes (Firestone and Davidson, 1989).

Experimental manipulation studies at the field scale, as well as studies along natural gradients, are valuable tools to understand how ecosystems will respond to climatic and environmental changes. While manipulation studies are well-suited to study effects of changes in specific experimentally controlled drivers, they are intrinsically constrained in time (years). Natural gradient studies are better suited to study effects on longer time scales (decades to centuries), but suffer from possible biases caused by changes in factors other than the gradient component under investigation, for example, climate. Studies based on a combination of experimental manipulations performed across multiple sites covering larger natural gradients should combine the strengths of both strategies and increase the reliability of the results.

Within the framework of the NitroEurope Integrated Project, we investigated how the soil-atmosphere exchange of the three greenhouse gases, CH_4, N_2O and CO_2, responded to simulated changes in drought, warming, drainage or nitrogen addition at nine experimental peatland and shrubland sites situated across natural gradients in precipitation and temperature. The aims of the study were (i) to identify environmental variables that correlated with GHG fluxes across the nine sites or within an ecosystem type, (ii) to evaluate whether specific variables had a direct effect on GHG fluxes and thereby could be identified as key drivers based on treatment responses at the site level, and (iii) to quantify the relative contribution of each GHG to the total global warming response for each experimental treatment.

2 Materials and methods

2.1 Study sites

The nine experimental peatland and shrubland sites included in the synthesis were all part of the NitroEurope project. The climatic conditions at the field sites ranged from subarctic conditions in northern Sweden to a Mediterranean climate in Spain, and from hydric temperate conditions in Wales to mesic temperate climates towards Estonia in the east (Fig. 1). The ecosystems represented open landscapes in these regions and included four peatlands and five shrublands (Table 1). The peatlands were all ombrotrophic bogs, while the shrublands covered heather-moorland (UK-Clo), dry *Calluna* heath (DK-Mol, DK-Bra, NL-Old) and Mediterranean garrigue shrubland (ES-Gar). Soil type and plant species

Table 1. Site characteristics.

Ecosystem and location	Coordinates	Altitude (m)	Site code	Soil type	Dominant vegetation[a]	Experimental treatment
Peatland						
Männikjärve, EE	58°52′ N 26°14′ E	80	EE-Män	Histosol	*S. fuscum, S. rubellum, Scheuchzeria palustris, Ledum palustre, Vaccinium oxycoccus, Chamaedaphne calyculata, Betula pubescencs, Pinus sylvestris*	Drainage
Storflaket, SE	68°20′ N 18°58′ E	380	SE-Sto	Histosol	*S. fuscum, S. balticum, E. nigrum, Andromeda polifolia, Rubus chamaemorus, E. vaginatum*	N addition (NH_4NO_3)
Fäjemyr, SE	56°15′ N 13°33′ E	140	SE-Fäj	Histosol	*C. vulgaris, Erica tetralix, S. magellanicum, S. rubellum, E. vaginatum*	N addition (NH_4NO_3)
Whim, UK	55°45′ N 03°16′ E	280	UK-Whi	Histosol	*C. vulgaris, E. vaginatum, S. capillifolium*	N addition (NH_4 or NO_3)
Shrubland						
Clocaenog, UK	53°03′ N 03°28′ W	490	UK-Clo	Peaty podzol	*C. vulgaris, Vaccinium myrtillus, E. nigrum*	Drought, warming
Mols, DK	56°23′ N 10°29′ E	57	DK-Mol	Sandy podzol	*C. vulgaris, Deschampsia flexuosa*	Drought, warming
Brandbjerg, DK	55°53′ N 11°58′ E	9	DK-Bra	Sandy podzol	*C. vulgaris, Deschampsia flexuosa*	Drought, warming
Oldebroek, NL	52°24′ N 05°54′ E	25	NL-Old	Sandy podzol	*C. vulgaris*	Drought, warming
Garraf, ES	41°19′ N 01°49′ E	210	ES-Gar	Petrocalcic calcisol	*Erica multiflora, Globularia alypum*	Drought, warming

[a] *C. vulgaris – Calluna vulgaris, E. nigrum – Empetrum nigrum, E. vaginatum – Eriophorum vaginatum, S. – Sphagnum.*

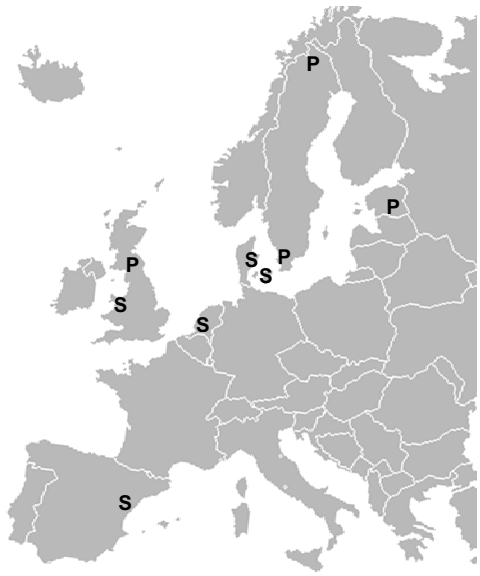

Fig. 1. Location of the nine experimental sites across Europe. Abbreviations are P for "peatland" and S for "shrubland".

composition differed between the sites, but all sites had dwarf shrubs in common, mainly *Calluna vulgaris*, *Empetrum nigrum* or *Erica* spp. (Table 1). The experimental sites existed before the NitroEurope project started and therefore already had ongoing methodologies and procedures.

2.2 Experimental manipulations

At the Estonian peatland EE-Män, the long-term effect of drainage was studied in a part of the bog where a drainage ditch system established in the 1950s (Veber, 1974) was reconstructed in 1975 (Table 1). Substantial lowering of the water table has resulted in significantly improved tree growth (Niinemets et al., 2001; Portsmuth et al., 2005). Thus, when this study took place, *Pinus sylvestris* was 10–12 m tall in the drained part of the bog compared to 1–3 m in the non-drained part. The effect of increased nitrogen deposition was investigated at three peatland sites. At the two Swedish sites SE-Sto and SE-Fäj, fertilized plots received NH_4NO_3 three times per year, corresponding to a total input of 4 g N m^{-2} yr^{-1} (Lund et al., 2009). At the Scottish peatland UK-Whi, treatment plots were given an extra 5.6 g N m^{-2} yr^{-1} in wet deposition using an automated sprinkler system, which provided ca. 120 artificial rain events per year coupled to rainfall (Sheppard et al., 2011). Nitrogen was applied either in the reduced form as NH_4Cl or in the oxidized form as $NaNO_3$. The effects of temperature increase and prolonged drought were tested at the five shrubland sites using passive night-time warming and horizontal rainout curtains, respectively. The experimental

setups at these sites are described in more detail by Beier et al. (2004) and Mikkelsen et al. (2008).

2.3 Soil greenhouse gas flux measurements

Fluxes of CH_4, N_2O and soil respiratory CO_2 were measured monthly or twice a month using static chamber methods described by, for example, Carter et al. (2011) and Sowerby et al. (2008). The chamber design and the measurement procedure varied across the nine sites. Overall, at least five weeks before simultaneous measurement of CH_4 and N_2O fluxes started, a permanent soil collar was installed in each replicated treatment plot ($n \geq 3$) on which a chamber was placed during measurements. Three to four times during the enclosure period of 20–180 min, a sample of headspace air was collected using a syringe through a septum in the chamber. At the Spanish shrubland ES-Gar, however, only two gas samples were collected during the 15 min enclosure period to avoid adverse effects on headspace air pressure in the small chambers (volume 0.8 l) used at this site. Gas samples were stored in plastic syringes (SE-Sto), pre-evacuated vials (UK-Clo, NL-Old) or non-evacuated vials using a double-needle approach (EE-Män, UK-Whi, DK-Mol, DK-Bra, ES-Gar) before analysis for CH_4 and N_2O concentrations by gas chromatography. One exception was the Swedish site SE-Fäj, where headspace concentrations of CH_4 and N_2O were determined in the field using a photoacoustic gas analyzer (Lund et al., 2009). Previous studies showed that gas flux rates measured using a photoacoustic gas analyzer were statistically identical to flux rates based gas sampling and analysis by gas chromatography (Ambus and Robertson, 1998; Lund et al., 2009). Soil CO_2 emissions were measured within permanent collars without vegetation, i.e. where any aboveground plant growth was removed on a regular basis. However, at the two Swedish sites SE-Sto and SE-Fäj, fluxes were assessed on vegetated plots and are therefore ecosystem respiratory CO_2 emissions. Generally, an opaque chamber was placed on the collar and the build-up of CO_2 during the enclosure period was monitored by a portable infrared gas analyzer. At three out of nine sites (EE-Män, NL-Old and ES-Gar), vascular plant free patches enabled soil CO_2 emissions to be determined concurrently with CH_4 and N_2O fluxes using the same chamber and gas chromatography approach. For all three gases, the flux rates were calculated using linear regression of headspace concentration versus time, except for long enclosure periods ($\geq$ 120 min), where CH_4 uptake was determined by fitting a first-order function.

The year of measurement deviated between sites, but was generally within the period 2006–2009. Annual cumulative fluxes were obtained by linear interpolation between measurement days. This is a common, but crude, upscaling procedure since measurements carried out at one point in time, and over a relatively short time span, will represent the whole period between two neighboring measurements, despite potentially large variation in the controlling climatic factors during this period. A more precise method would be to model the fluxes based on more frequent measurements of key drivers such as temperature and soil moisture (e.g. Selsted et al., 2012). In the present study, we did not have sufficient measurements to build such a model for each site. At the Swedish peatlands SE-Fäj and SE-Sto, gas flux measurements were not conducted during the five and eight coldest months of the year, respectively. When calculating the annual CO_2 fluxes for these two sites, we assumed that the contribution of the winter period to annual fluxes is 9 % at SE-Fäj (Lindroth et al., 2007) and 22 % at SE-Sto (Larsen et al., 2007). These ratios were also used to estimate annual CH_4 and N_2O fluxes, assuming that CO_2 fluxes represented the general level of microbial activity; more specifically, that Q_{10} values for CO_2, CH_4 and N_2O fluxes were not substantially different. Reported Q_{10} values for CH_4 and N_2O fluxes in natural ecosystems are sparse, however, Smith (1997) compiled Q_{10} values for N_2O emissions ranging 2.8–3.1 and 1.5–5 in unfertilized grassland and short grass prairie, respectively. This is within the range of Q_{10} values often reported in literature for soil respiratory CO_2 emissions (e.g. Kim and Verma, 1992).

2.4 Precipitation, temperature and soil moisture

The meteorological measurements included precipitation and air temperature. Soil temperature probes were installed at either 5 cm depth (EE-Män, SE-Fäj, DK-Bra, ES-Gar) or 10 cm depth (UK-Whi, UK-Clo, DK-Mol, NL-Old) and data were logged at least twice a day. Volumetric soil water content was measured at least twice a week by time domain reflectometry probes installed in the top 6 cm (DK-Mol), 10 cm (SE-Sto, SE-Fäj, UK-Clo, NL-Old), 15 cm (ES-Gar) or 20 cm soil layer (DK-Bra). For UK-Whi and EE-Män, volumetric soil moisture in 0–10 cm depth was calculated from monthly or seasonal measurements of gravimetric soil water content. At the peatland sites, water table depth was measured manually in dipwells or automatically using a pressure transducer.

2.5 Nitrogen deposition and leaching

Bulk N deposition was measured with bulk deposition collectors with a minimum diameter of 10 cm. Concentrations of NH_4^+, NO_3^- and total N in the sampled precipitation were determined by ion chromatography or colorimetric assays. If bulk N deposition was not assessed at the site, then total N deposition reported in the literature for this specific area was used instead. Nitrate leaching was determined from modelled water percolation combined with NO_3^- concentrations measured in soil water collected below the rooting zone using porous suction cups (Schmidt et al., 2004; Larsen et al., 2011). Soil water was also sampled in the upper part of the soil profile, generally just below the O-horizon, and analyzed for NH_4^+ and NO_3^- concentrations (e.g. Schmidt et al., 2004).

2.6 Plant and soil analyses

Aboveground biomass C and N at the shrubland sites were determined using plant tissue C and N analyses combined with biomass estimation based on pinpoint data (Peñuelas et al., 2004; Beier et al., 2009). Briefly, pinpoint measurements were conducted by lowering a sharpened pin through the vegetation, recording species and height for each plant hit with the pin. Total plant biomass was subsequently estimated using a site specific correlation between pinpoint data and actual biomass obtained by destructive samplings outside the experimental plots (Peñuelas et al., 2004). At the Scottish peatland UK-Whi, biomass C and N were obtained by destructive harvest. Soil samples collected in the upper soil layer (i.e. generally the O-horizon) were extracted with distilled water (DK-Bra), 1 M KCl (UK-Whi, UK-Clo, DK-Mol, NL-Old) or 2 M KCl (EE-Män, ES-Gar) to measure concentrations of extractable NH_4^+ and NO_3^- by ion chromatography or colorimetric assays (e.g. Andresen et al., 2009). For upper and lower soil layers, soil pH was determined in a suspension of soil in distilled water, 0.01 M $CaCl_2$ or 1 M KCl. Furthermore, bulk density, total C and N contents, and microbial biomass C were measured as described by Beier et al. (2009), and C stock in the 0–10 cm soil layer was calculated.

2.7 Responsiveness

Relative responsiveness (RR, %) of CH_4, N_2O and CO_2 fluxes to the different treatments was calculated as

$$\mathrm{RR} = (\mathrm{Treat} - \mathrm{Cont})/\mathrm{Cont} \times 100 \quad (1)$$

where Treat is mean treatment flux across sites and Cont is mean control flux across sites (given in mg C or N m^{-2} yr^{-1}). Similar to Liu and Greaver (2009), we refrained from calculating relative responsiveness in cases where the flux changed direction in response to a treatment. This occurred in the N addition experiments, where N_2O fluxes changed from net uptake to emission. For each gas species and treatment, we also calculated the absolute responsiveness (AR) in CO_2-equivalents as

$$\mathrm{AR} = (\mathrm{Treat} - \mathrm{Cont})/\mathrm{MR} \times \mathrm{GWP} \quad (2)$$

where molar ratio (MR) is 12 / 16, 28 / 44 and 12 / 44, and global warming potential (GWP) is 25, 298 and 1 for CH_4, N_2O and CO_2, respectively (IPCC, 2007b). To compare the relative contribution of each gas species to the total global warming response for a specific treatment, we calculated the fractional importance (FI, %) of each gas species as

$$\mathrm{FI}_i = |\mathrm{AR}_i|/(|\mathrm{AR}_1| + |\mathrm{AR}_2| + |\mathrm{AR}_3|) \times 100 \quad (3)$$

where $|AR_1|$ to $|AR_3|$ is the numerical value of the absolute responsiveness for each of the three gases. Numerical values were used in order for the total global warming response of all three gases to sum up to 100. To clarify, when assessing the overall response patterns across sites and treatments, we calculated RR, AR and FI for all treatments, also including treatments that did not show significant effects on flux rates in the cross-site statistical analysis described below.

2.8 Statistics

To assess treatment effects on GHG fluxes across the sites, two-factor analyses of variance (ANOVA) with treatment, site and their interaction as fixed effects were conducted using the PROC MIXED procedure of SAS (SAS Institute, 2003). The random effects included site and treatment × site. The GHG flux datasets were unbalanced as the number of replicates was unequal between sites, however the MIXED procedure is used in the same way whether data are balanced or unbalanced (Littell et al., 2002). More specifically, equal weight is given to each site regardless of the number of replicates when determining treatment effects. Data were log-transformed as required to obtain normality and homogeneity of variance. Main effects and interactions with $P \leq 0.05$ were considered to be significant, and were interpreted using differences of least squares means.

3 Results

3.1 Precipitation, temperature and soil moisture

Annual precipitation during the study period ranged from 311 mm at the subarctic peatland Storflaket in Sweden (SE-Sto) to 1351 mm at the Welsh shrubland Clocaenog (UK-Clo) (Table 2). Mean annual air temperature in control plots ranged from −0.3 °C at the subarctic site SE-Sto to 15.6 °C at the Spanish shrubland Garraf (ES-Gar). The passive nighttime warming generally raised annual soil temperature at 5 or 10 cm depth by 0.5 °C, and decreased the soil water content by 2 % vol. (Table 2). Experimental drought caused a decline in mean annual soil moisture ranging from a reduction of 1.5 % vol. at the Danish shrubland Brandbjerg (DK-Bra) to 10.2 % vol. at the wet shrubland UK-Clo. Drainage of the Estonian peatland Männikjärve (EE-Män) lowered the water table from an annual depth of 15 cm to 38 cm below soil surface. Characteristics of the upper and lower soil layers at the sites as well as data on aboveground biomass, N deposition and leaching are found in the Supplement.

3.2 CH_4 fluxes

Annual CH_4 fluxes in control plots ranged from uptake of 519 mg C m^{-2} yr^{-1} at the Danish shrubland Mols (DK-Mol) to emission of 6890 mg C m^{-2} yr^{-1} from the Scottish peatland Whim (UK-Whi) (Table 3). For the peatland sites, the natural gradient analysis showed a clear relationship between annual CH_4 emission and mean annual air temperature (Fig. 2). In contrast, CH_4 fluxes at the temperate shrublands

Table 2. Annual precipitation, air temperature (Air T), soil temperature (Soil T) and soil water content (Soil W) during the study period at the nine experimental sites depending on treatment.

Site	Treatment	Precipitation (mm)	Air T (°C)	Soil T (°C)	Soil W (% vol)
EE-Män	Control	889	4.3	5.6	76.7
	Drainage	na	6.0	5.9	37.2
SE-Sto	Site data	311	-0.3	na	23.4
SE-Fäj	Site data	626	7.1	7.6	59.1
UK-Whi	Site data	1092	8.5	7.7	71.9
UK-Clo	Control	1351	7.5	8.1	45.9
	Drought	1130	7.7	7.9	35.7
	Warming	1127	7.9	8.1	43.6
DK-Mol	Control	668	8.7	9.0	17.6
	Drought	563	8.9	9.0	15.1
	Warming	na	9.7	9.8	15.3
DK-Bra	Control	714	10.2	8.9	16.5
	Drought	na	na	8.8	15.0
	Warming	na	na	9.4	15.0
NL-Old	Control	986	8.3	8.8	21.8
	Drought	792	na	na	17.1
	Warming	855	9.6	9.5	16.5
ES-Gar	Control	550	15.6	17.0	19.8
	Drought	397	15.8	16.8	17.0
	Warming	520	16.1	17.7	20.0

na: not assessed.

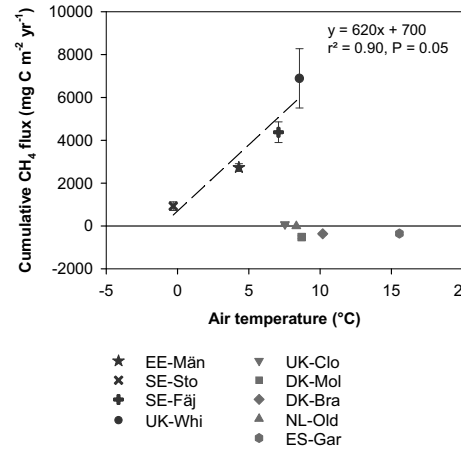

Fig. 2. Annual CH_4 flux related to mean annual air temperature in control plots at the nine sites (means ± SE). A linear regression line was fitted to data from the four peatland sites. Site codes are described in Table 1.

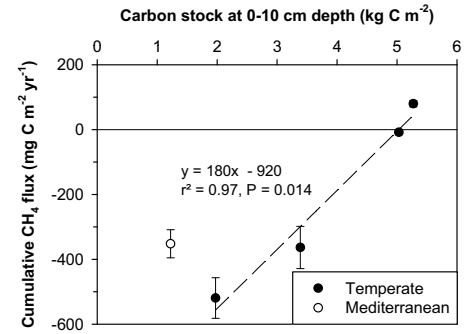

Fig. 3. Annual CH_4 flux as a function of carbon stock in the 0–10 cm soil layer at five shrubland sites (means ± SE). A linear regression line was fitted to data from the four temperate shrubland sites.

correlated with the carbon stock in the top 10 cm soil layer (Fig. 3). Concerning experimental manipulations, the effect of increased N input was tested at three peatland sites and overall no uniform effect was found on the CH_4 efflux ($P = 0.65$) (Table 3). At the peatland UK-Whi, though, additional deposition of oxidized N, corresponding to 5.6 g NO_3-N m^{-2} yr^{-1}, resulted in an almost 300 % increase of the annual CH_4 emission ($P = 0.031$). Concurrently, soil pH rose from 3.7 to 4.0 (Table S1). Permanent drainage of the Estonian peatland EE-Män reduced the CH_4 efflux substantially ($P = 0.003$), whereas no consistent effect on the CH_4 flux was observed for prolonged drought or warming at the shrubland sites ($P \geq 0.49$) (Fig. 4, Table 3).

3.3 N_2O fluxes

Annual fluxes of N_2O generally consisted of low efflux rates, although net N_2O uptake was observed in control plots at the Swedish peatland Fäjemyr (SE-Fäj) and the Scottish peatland UK-Whi (Table 3). Within each of the two ecosystem types, peatland and shrubland, the highest N_2O efflux occurred at the site with highest soil water NO_3^- concentration (Fig. 5). The stimulatory effect of NO_3^- on the N_2O efflux was supported by the N deposition experiments. More specifically, the two peatlands SE-Fäj and UK-Whi changed from N_2O sinks into N_2O sources as a result of increased NH_4NO_3 and $NaNO_3$ input, respectively ($P = 0.011$) (Fig. 6a). Overall, differences in volumetric soil water content across sites did not explain the variation in annual N_2O fluxes for control plots (Fig. 6b). At the site level, however, reduced soil moisture caused by drainage or drought consistently reduced the N_2O efflux ($P = 0.0005$). According to differences of least squares means, this was especially pronounced for drainage at the Estonian peatland EE-Män and for drought at the Dutch shrubland Oldebroek (NL-Old) (site × treatment; $P = 0.034$). The warming treatment had no effect on N_2O fluxes ($P = 0.47$) (Table 3).

3.4 Soil respiratory CO_2 emission

Soil CO_2 emissions ranged between 518 to 732 g C m^{-2} yr^{-1} at six of the nine sites investigated (Fig. 7a), but was considerable lower at the remaining three sites. As expected, the lowest rate was observed at the northernmost and coldest site SE-Sto (231 g C m^{-2} yr^{-1}), where fluxes were only measured on vegetated plots and therefore are ecosystem-level respiratory CO_2 losses. Consequently, soil respiratory CO_2 losses should be even lower than the rate reported here. Soil respiration was also considerably lower at the temperate shrubland NL-Old (310 g C m^{-2} yr^{-1}), and at the southernmost and warmest shrubland ES-Gar (390 g C m^{-2} yr^{-1}), compared to the six sites with highest emissions. Across the five sites where warming was applied as treatment, the temperature rise of 0.5 °C in the soil had no effect on soil CO_2 emissions ($P = 0.83$) (Table 3). At the site level, the CO_2 efflux seemed to be more controlled by soil moisture. More specifically, prolonged drought and long-term (> 30 yr) drainage overall caused a reduction of soil CO_2 emissions ($P = 0.003$) (Fig. 7b). In particular, this was pronounced for drainage at the peatland EE-Män and drought at the dry shrubland DK-Mol, whereas drought tended to have the opposite effect at the wet shrubland UK-Clo (site × treatment; $P = 0.0086$). Increased N input at three peatland sites had no effect on annual CO_2 emissions ($P = 0.69$) (Table 3).

3.5 Responsiveness of greenhouse gas fluxes

Relative to control plots, the observed treatment response was generally higher for CH_4 and N_2O fluxes than for CO_2 fluxes (Table 4). However, when comparing the fractional importance of each GHG to the total numerical global warming response (GWR), the changes in CO_2 emissions dominated the response in all treatments (ranging 71–96 %), except for NO_3^- additions where 89 % of the total GWR was due to a change in CH_4 emissions. Relative to the other GHGs, the impact on GWR from changes in N_2O fluxes was generally low; only 4 % of total GWR across the shrubland sites and absent across the peatland sites. Across all treatments and gas species, anthropogenic disturbance overall reduced the shrublands' contribution to global warming, while peatlands generally showed an increased contribution for CH_4 and a reduced contribution for CO_2.

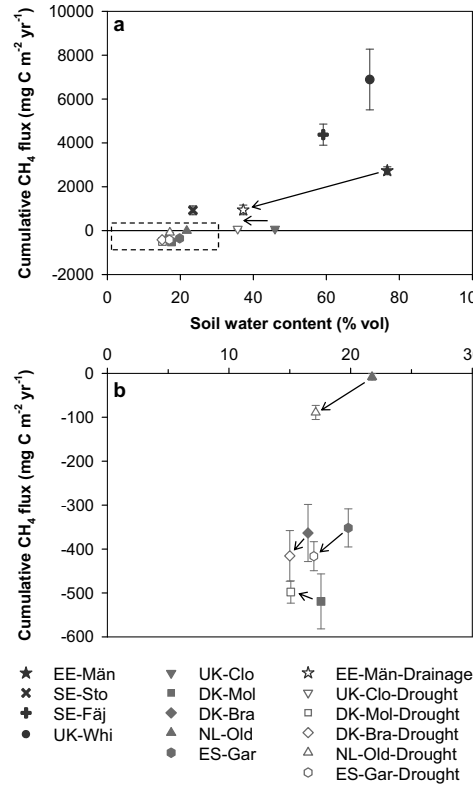

Fig. 4. Annual CH_4 flux at the nine sites related to mean annual soil water content, predominantly measured in the top 10 cm soil layer (means ± SE). Closed symbols are control plots, whereas open symbols are drained or drought treated plots. Arrows indicate direction of treatment responses. In **(b)**, the bottom left corner of **(a)** has been enlarged. Site codes are described in Table 1.

4 Discussion

In Europe, open landscapes represented by peatlands and shrublands cover about 7 % of the land area (EEA, 2006; Montanarella et al., 2006). In the current synthesis, we investigated greenhouse gases fluxes between these ecosystems and the atmosphere in order to identify factors that seemed to control cross-site variation in flux rates, either directly or indirectly. Furthermore, we assessed the effect on the GHG fluxes of anthropogenic disturbances, such as temperature rise and prolonged droughts caused by climate change, drainage of peatlands and increased atmospheric nitrogen deposition.

4.1 CH_4 fluxes

On a global scale, wetlands are the largest single source of CH_4 emission to the atmosphere, accounting for 100 to 231 Tg CH_4 yr^{-1} (IPCC, 2007b), whereas aerobic soils are substantial CH_4 sinks, estimated to take up about 22 Tg CH_4 yr^{-1} (Dutaur and Verchot, 2007). The present study illustrates these fundamental differences between

Table 3. Annual cumulative CH_4, N_2O and soil CO_2 fluxes at the nine experimental sites depending on treatment (means; SE in brackets). Number of replicated plots and year of measurement (either one or two years) are also indicated.

Site	Treatment	Replicates	CH_4 flux mg C m^{-2} yr^{-1}		N_2O flux[a] mg N m^{-2} yr^{-1}		Year	Soil CO_2 emission[b] g C m^{-2} yr^{-1}		Year
EE-Män	Control	3	2729	(180)	41.8	(5.7)	2008/2009	528	(66)	2008/2009
	Drainage		943	(222)	4.1	(7.9)		226	(56)	
SE-Sto	Control	4	926	(201)	na		2007	231	(6)	2007
	NH_4NO_3		551	(110)	na			265	(39)	
SE-Fäj	Control	4	4377	(482)	−2.4	(1.8)	2007	522	(179)	2007
	NH_4NO_3		5883	(846)	8.0	(3.7)		585	(51)	
UK-Whi	Control	4	6890	(1383)	−14.3	(3.5)	2007–2009	572	(69)	2006/2007
	NH_4		7412	(2590)	5.5	(16.6)		535	(51)	
	NO_3		27 020	(11 101)	16.1	(13.2)		496	(26)	
UK-Clo	Control	3	80	(10)	10.8	(5.7)	2007–2009	518	(32)	2006/2007
	Drought		81	(12)	0.8	(2.7)		633	(12)	
	Warming		70	(7)	11.2	(2.1)		584	(45)	
DK-Mol	Control	3	−519	(63)	13.3	(5.7)	2006/2007	732	(36)	2003/2004
	Drought		−498	(25)	9.9	(4.6)		513	(18)	
	Warming		−552	(36)	10.8	(2.3)		730	(48)	
DK-Bra	Control	6	−363	(65)	31.0	(5.6)	2006/2007	651	(59)	2006/2007
	Drought		−416	(58)	27.6	(7.8)		600	(34)	
	Warming		−436	(78)	29.5	(6.5)		659	(52)	
NL-Old	Control	3	−8	(7)	6.9	(5)	1999	310	(48)	1999
	Drought		−89	(16)	−30.4	(10.2)		285	(72)	
	Warming [c]		29	(20)	−2.3	(2.3)		330	(45)	
ES-Gar	Control	3	−352	(43)	na		2007/2008	390	(30)	2007/2008
	Drought		−416	(33)	na			298	(19)	
	Warming		−432	(42)	na			335	(21)	

[a] Fluxes of N_2O were not assessed (na) at SE-Sto and ES-Gar.
[b] Data for SE-Sto and SE-Fäj is ecosystem respiratory CO_2 emission.
[c] At NL-Old, the warming treatment started in May 1999.

Table 4. Relative responsiveness to treatments compared to controls (%), absolute responsiveness (CO_2-equivalents), and fractional importance of each greenhouse gas to the total numerical global warming response (in brackets, %) for soil CH_4, N_2O and CO_2 fluxes across all sites.

Treatment[a]	Relative responsiveness (%)			Absolute responsiveness (CO_2-eq)					
	CH_4	N_2O[b]	CO_2	CH_4		N_2O		CO_2	
Drainage	−65	−90	−57	−74	(6)	−18	(2)	−1107	(92)
N addition, including NH_4	14	na	5	23	(22)	7	(7)	73	(71)
N addition, including NO_3	174	na	2	295	(89)	10	(3)	25	(8)
Drought	−15	−87	−11	−1	(1)	−6	(3)	−201	(96)
Warming	−14	−21	1	−1	(4)	−1	(5)	27	(91)
Peatlands all treatments	124	0.4	−9	193	(56)	0	(0)	−153	(44)
Shrublands all treatments	−14	−54	−5	−1	(2)	−4	(4)	−87	(94)

[a] Responsiveness is reported for the treatments "warming" and "N addition, including NH_4" although cross-site statistical analyses showed no significant effects on any of the gas species.
[b] na: not assessed due to change in flux direction.

ecosystem types depending on water table depth with peatlands as CH_4 sources and shrublands generally as CH_4 sinks. The CH_4 emission from peatlands correlated positively with temperature when analyzed across the four sites. Although our natural gradient analyses could be biased by differences in measurement years and methods, the results are in line with Christensen et al. (2003) who compared CH_4 fluxes measured during the growing season at five northern wetlands covering Greenland, Iceland, Scandinavia and Siberia. They estimated that soil temperature explained 84 % of the

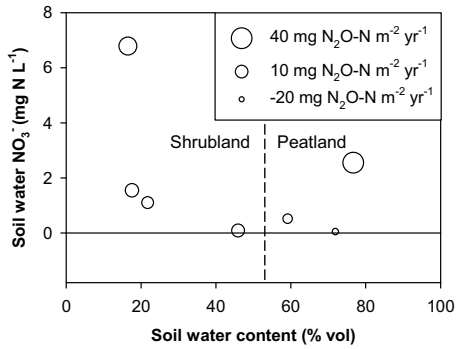

Fig. 5. Size of annual N_2O flux plotted against mean annual soil water content and nitrate concentration in soil water. Data derive from control plots at seven sites that are divided into shrublands and peatlands by the dashed line.

variability in mean seasonal CH_4 emissions, and suggested that availability of organic acids in peat water accounted for the remaining 16 % of the CH_4 fluxes variability across the sites. The stimulating effect of increased temperature on net CH_4 emissions from peatlands can be explained by basic CH_4 biochemistry as processes related to CH_4 production are more temperature dependent than those associated with methane oxidation (Dunfield et al., 1993). However, the simple temperature relationship of CH_4 emissions may also include other interrelated factors, most importantly plant productivity affecting substrate quality and quantity, but also plant aerenchyma development allowing CH_4 produced in the soil to be released into the atmosphere (Joabsson et al., 1999). Based on the current dataset, we are not able to distinguish whether temperature affected the CH_4 efflux directly or indirectly, and most likely several of the temperature-related mechanisms above acted simultaneously. Nevertheless, our natural gradient analysis suggests that temperature could be used as a common indicator when estimating the magnitude of CH_4 emissions from peatlands across wide regional scales. Furthermore, the analysis reveals that CH_4 emissions from peatlands may rise in the future as a result of the predicted temperature increases caused by climate change.

In a global analysis of CH_4 uptake by aerobic soils, Dutaur and Verchot (2007) found that soil texture strongly controlled CH_4 uptake with coarse textured soils consuming more CH_4 than other texture classes. All temperate shrublands in our study had a sandy mineral soil layer, while the soils differed in the properties of the organic layer, which consisted of peat at the heather-moorland (UK-Clo) and of mor humus at the dry *Calluna* heaths (DK-Mol, DK-Bra, NL-Old). Net CH_4 uptake at the four temperate shrublands correlated negatively with the carbon stock in the upper 10 cm soil layer, suggesting a switch from net CH_4 uptake to net CH_4 emission as soils become enriched in organic matter. To our knowledge, such a relationship has only been reported in very few studies. For instance, Singh et al. (1997) showed that CH_4 uptake in dry tropical forest soils correlated with both carbon and nitrogen content of the soils; two variables which increased in parallel. Within soil types, the content of organic matter and soil water often correlate, either because organic matter increases the water holding capacity (Hudson, 1994) or because high soil moisture hinders aerobic decomposition (Jungkunst and Fiedler, 2007). In line with this, the two sites with the highest carbon stock of the four temperate shrublands (i.e. UK-Clo and NL-Old) also had the highest soil moisture (Table 2). Consequently, the apparent carbon control on net CH_4 exchange could be indirect via an effect on soil moisture, which limits CH_4 diffusion towards the zone of methanotrophic activity (King, 1997; Dunfield, 2007). Singh et al. (1997), however, suggested a more direct control mechanism, where reduced net uptake of CH_4 in carbon-rich soils was due to decreased O_2 content in the soil atmosphere, resulting from higher decomposition rates. In our study, though, we found no relationship between carbon stock and soil respiratory CO_2 emission at the four temperate shrubland sites. Potentially, organic matter stimulated methanogenesis by acting as a substrate for the process, leading to lower net CH_4 uptake in the carbon-rich soils in our study. Most likely, the correlation between soil organic C and CH_4 uptake was caused by a combination of these indirect and direct mechanisms, and we suggest that future studies aim at investigating this relationship in more details.

Previous studies of N additions to wetlands reported both positive and negative responses in net CH_4 emissions, and the mechanisms involved are still under debate (Bodelier, 2011). In the present study, $NaNO_3$ addition at the Scottish bog UK-Whi increased the CH_4 efflux by almost 300 %, whereas NH_4Cl or NH_4NO_3 additions had no overall effect. At the Scottish bog, the addition of $NaNO_3$ raised pH in peat water from 3.7 to 4.0 (Table S1) and increased the availability of dissolved organic carbon (DOC), possibly because the slightly higher pH enhanced the solubility of organic compounds in the soil (Evans et al., 2008). In contrast, NH_4Cl addition had no effect on pH or DOC. For comparison, Murakami et al. (2005) raised the pH of four tropical acid peat soils by ca. 2 pH units and observed a vast increase in the CH_4 production potential. The authors suggested that this was due to increased substrate supply for methanogenic microorganisms derived from decomposed organic matter such as organic acids from peat humus. A similar mechanism could explain the enhanced CH_4 efflux in response to $NaNO_3$ addition at the Scottish bog UK-Whi.

At the Swedish peatland sites SE-Fäj and SE-Sto, addition of NH_4NO_3 had no significant effects on the CH_4 flux. Although insignificant, the opposing effects of N addition on CH_4 fluxes at SE-Fäj (increased efflux) and SE-Sto (decreased efflux), illustrates the importance of peatland type and site specific properties, such as plant composition and

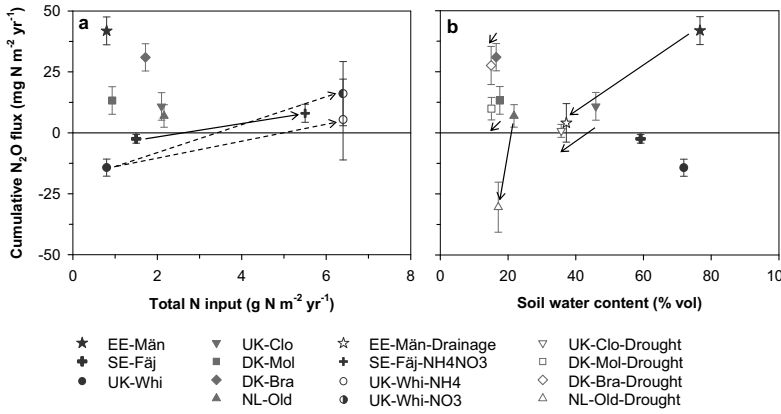

Fig. 6. Annual N_2O flux at seven sites related to atmospheric N deposition **(a)** and mean annual soil water content, predominantly measured in the top 10 cm soil layer **(b)** (means ± SE). In addition to data for control plots, results are presented for plots that were exposed to either increased N input **(a)** or drainage and drought **(b)**. Arrows indicate direction of treatment responses. Site codes are described in Table 1.

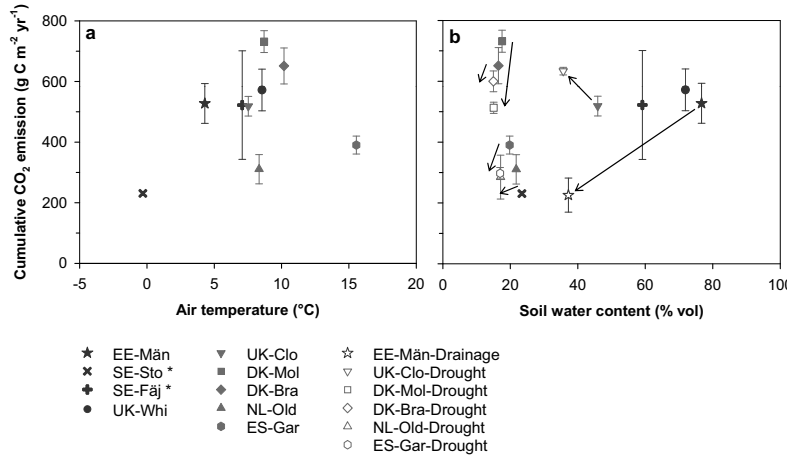

Fig. 7. Annual soil respiratory CO_2 emission in control plots at the nine sites related to mean annual air temperature **(a)** and mean annual soil water content, predominantly measured in the top 10 cm soil layer **(b)** (means ± SE). In **(b)**, data for drained or drought treated plots are also presented with arrows indicating direction of treatment responses. * Data for SE-Sto and SE-Fäj is ecosystem respiratory CO_2 emission. Site codes are described in Table 1.

productivity, pH, substrate quality and general hydrology, for the effects of increased nutrient availability on peatland CH_4 fluxes (Keller et al., 2006).

4.2 N_2O fluxes

Latest advances in knowledge suggest that three separate processes contribute to N_2O emissions from soils. These are nitrification, denitrification and dissimilatory nitrate reduction to ammonium (DNRA), which are thought to be important sources of N_2O in oxic, sub-oxic and anoxic microsites of the rhizosphere, respectively (Baggs, 2011). At individual field sites, daily N_2O emission rates are typically reported to increase with soil moisture in the range from 50 to 90 % water filled pore space (WFPS) (Smith et al., 2003). This

relationship is probably caused by the associated decrease in O_2 diffusion within the soil, leading to larger anaerobic microsites and thereby increased N_2O production via denitrification (Smith et al., 2003) and possibly also DNRA. However, when soil moisture exceeds 90 % WFPS, N_2O emissions may decline as N_2 is the final product of denitrification in most waterlogged soils (Davidson, 1991), although pH may also influences the N_2O / N_2 loss ratio of denitrification (Šimek and Cooper, 2002).

In the present study, the lack of relationship between N_2O emission and volumetric soil moisture across sites was possibly because N_2O production is controlled by gas diffusivity, which is poorly reflected by volumetric soil moisture when comparing soils that differ in total porosity. At the site level, however, net N_2O emissions declined consistently in response to reduced soil moisture caused by drought treatment, supporting previous results from drought experiments in a spruce forest (Goldberg and Gebauer, 2009). Furthermore, N_2O emissions from the Estonia peatland EE-Män declined due to long-term drainage, which is in contrast to results from previous drainage experiments in ombrotrophic bogs (Martikainen et al., 1993; Regina et al., 1996). Apparently, increased tree growth at the drained area reduced the availability of soil nitrate (Table S1), which in combination with lower soil moisture caused the decline in N_2O emissions.

Analysed across sites, the shrubland and peatland with highest NO_3^- concentration in soil water also had the highest annual N_2O emission within each ecosystem type, which illustrates that N_2O production in these nutrient poor ecosystems was limited by N availability. This was supported by data at the site level, where N additions ($< 60\,kg\,N\,ha^{-1}\,yr^{-1}$) that included nitrate led to increased N_2O efflux from two peatlands, suggesting that denitrification or DNRA were involved in N_2O production in these ecosystems, and that the processes were stimulated by increased substrate availability.

4.3 Soil respiratory CO_2 emission

Annual soil respiratory CO_2 emissions (R_s) ranged from 231 to $732\,g\,C\,m^{-2}\,yr^{-1}$ in control plots across the nine sites. The highest fluxes were observed at the temperate sites with annual temperatures ranging 8–10 °C, where ecosystem C turnover was less likely to be inhibited by either low temperatures (i.e. SE-Sto) or low summer soil moisture, resulting from low precipitation combined with high temperatures (i.e. ES-Gar). For comparison, Hibbard et al. (2005) reported R_s rates of 427–$1805\,g\,C\,m^{-2}\,yr^{-1}$ across evergreen temperate forests, 509–$867\,g\,C\,m^{-2}\,yr^{-1}$ in temperate/Mediterranean broadleaved forests and 181–$488\,g\,C\,m^{-2}\,yr^{-1}$ in temperate/Mediterranean woodlands and savannas. The low fluxes at ES-Gar and NL-Old may be biased by the method applied, where headspace CO_2 concentrations were determined by gas chromatography together with CH_4 and N_2O. This implied longer enclosure periods, i.e. 15 or 60 min compared to 1–5 min when measured directly in the field using an infrared gas analyzer. Longer enclosure periods may lead to non-linear development of headspace CO_2 concentrations over time, and subsequently to underestimation of flux rates if calculations are based on linear regression (Heinemeyer and McNamara, 2011). However, EE-Män had the fourth highest CO_2 flux among the nine sites and here fluxes were also determined using the gas chromatography method. Furthermore, Beier et al. (2009) estimated annual soil respiration at ES-Gar to be $440\,g\,C\,m^{-2}\,yr^{-1}$ based on other data with shorter enclosure periods (4 min), which still is lower than our estimates for six of the temperate sites. The low flux at the Dutch shrubland NL-Old could be due to the vegetation which, unlike the other sites, is a *Calluna* monoculture (Table 1). Van Vuuren and van der Eerden (1992) found that *Calluna* litter had a lower decomposition rate compared to litter of *Deschampsia flexuosa* and *Molinia caerulea*, which coincided with a double concentration of recalcitrant carbon compounds, indicated by the Klason lignin content. The low CO_2 efflux at NL-Old could therefore result from a lower quality of the soil C pool compared to the other sites. Thus, our results indicate a pattern across the sites where soil respiration increased with temperature, but that the increase was offset in the warmest ecosystem due to low soil moisture, resulting from low precipitation combined with high temperatures. At the site level, however, warming treatment had no overall effect on soil respiratory CO_2 losses, which may be due to the relatively mild temperature increase achieved with the passive warming technique of 0.5 °C in the soil.

The prolonged drought or drainage manipulations showed stronger effects on soil respiration rates than both warming and N additions. Reduced soil moisture due to drought or drainage generally decreased soil respiration rates at the dry shrublands and the wet, long-term drained peatland EE-Män, but increased soil respiration rates at the wet shrubland UK-Clo. As drainage most often leads to increased CO_2 emissions in hydromorphic soils, at least in the short-term (Jungkunst and Fiedler, 2007; Danevčič et al., 2010; Maljanen et al., 2010), our results highlight how results may differ depending on the time since the experimental treatments were initiated. The drainage treatment at EE-Män started more than 30 yr ago. Currently, the easy degradable fractions of the peat at EE-Män have already been decomposed and carbon cycling in the ecosystem has reached a new balance. In contrast, the drought treatment at UK-Clo has only been running for eight years, thus it is likely that the long-term effect may differ from the current short-term effect. Furthermore, the different response directions to prolonged drought between the sites show how important the initial conditions are for the strength and directions of the response to the treatment.

4.4 Experimental manipulations

The quantitative effect on GHG fluxes of an experimental treatment is to a certain extent controlled by the magnitude of the treatment effects on environmental drivers. The question is whether our experimental manipulations have caused realistic changes in these drivers, which enable us to predict changes in GHG fluxes in future? On an annual basis, the prolonged drought treatment at the shrubland sites reduced moisture in the top soil by 10–20 %, which is a consistent decline when taking into account that the drought treatment was only carried out for about two months per year. In contrast, the warming treatment generally raised annual soil temperature at the sites by 0.5 °C. For comparison, annual mean soil temperatures in control plots at the Welsh shrubland UK-Clo varied between 7.0 and 8.7 °C during the years 2000–2007 (data not shown). Thus, the overall warming effect of 0.5 °C was less than the interannual variability at the site level. Furthermore, surface temperatures in Europe are projected to increase 2–5 °C during this century, depending on which scenario is used (IPCC, 2007a). In conclusion, the lack of experimental warming effect on any of the three GHGs in our study could relate to an insufficient temperature increase obtained by the passive night-time warming treatment.

By contrast, drainage about 30 yr ago of the Estonian peatland EE-Män has more than doubled the size of the oxic zone by lowering the water table from an annual depth of 15 cm to 38 cm below soil surface. As a result, the emission of all three GHGs had dropped dramatically after 30 yr of treatment. Furthermore, annual N deposition was raised from 0.2, 1.5 and 0.8 g N m^{-2} to 4.2, 5.5 and 6.4 g N m^{-2} at the peatlands in northern (SE-Sto) and southern Sweden (SE-Fäj) as well as Scotland (UK-Whi), respectively. It is unlikely that total inorganic N deposition in these regions of Europe will reach such high levels in future (Galloway et al., 2004). However, global warming will lead to increased soil mineralization rates, releasing more inorganic N and other nutrients for plant uptake (Rustad et al., 2001; Mack et al., 2004). Also, large regions of South Asia are projected to receive more than 5 g N m^{-2} yr^{-1} by 2050. Apart from the CH_4 response to $NaNO_3$ addition at the Scottish peatland UK-Whi, responses in GHGs fluxes to these relatively high N application rates were minor, illustrating resilience in the peatland ecosystems towards increased N deposition in the short term. However, in the longer term, changes in peatland plant composition and structure may occur, affecting the exchange of greenhouse gases. For instance, a warmer and drier climate with increased nutrient turnover will inflict competitive disadvantages for *Sphagnum* mosses, as they are adapted to cold, wet and nutrient-poor conditions (Clymo, 1984), and possibly stimulate growth of vascular plants.

4.5 Responsiveness

We observed some clear and interesting patterns in the responses of the three GHG fluxes across the nine investigated ecosystems. First of all, the observed responses to the manipulations of climate and atmospheric N deposition were stronger in relative terms for CH_4 and N_2O fluxes than for soil CO_2 emissions. However, when comparing the fractional importance of each GHG to the total global warming response, then the observed changes in soil CO_2 efflux dominated the response for the drainage, drought and warming treatments, where at least 91 % of the global warming response was caused by changes in soil CO_2 fluxes. The N addition treatments in peatlands that included NO_3^- differed from this overall pattern by the dominance of a change in CH_4 emissions (89 % of global warming response) compared to changes in the other gas species. This profound effect therefore also partly explains why responses in CH_4 fluxes played a slightly more dominant role across the four peatlands (56 % of the global warming response) compared to soil CO_2 emissions (44 % of global warming response) and N_2O fluxes, which did not contribute significantly. In contrast, across the five shrublands including both drought and warming treatments, the response in soil CO_2 emissions dominated (94 % of global warming response) by far over responses in CH_4 fluxes (2 % of global warming response) and N_2O fluxes (4 % of global warming response).

Based on 109 different studies on effects of N addition on ecosystem GHG budgets, Liu and Greaver (2009) reported an overall increase in the terrestrial CO_2 sink, but also that this was largely offset (53–76 %) by stimulated CH_4 and N_2O emissions. When adding up the absolute treatment responses for the three GHGs in our study, the N addition experiments led to a substantial positive climate forcing response. However, this was caused by a strong increase in CH_4 emissions, whereas Liu and Greaver (2009) found that N_2O was the primary contributor to global warming across the studies in their review. Warming effects were very small and not significant in the overall cross-site ANOVA. By contrast, the drought and drainage treatments led to a uniformly negative climate forcing response. Again, it must be emphasized that the response for the drained peatland in this study is the long-term response and it is likely that a substantial amount of carbon was lost from the ecosystem in the years immediately after drainage. But at least across the five shrubland ecosystems, the drought treatment showed a clear pattern of negative feedback to climate change.

In general, we recognize that the nine sites included in this study span differences in soil types, plant species, pretreatment conditions and present climate, and that the experimental treatments differed between sites. For this reason, generalized conclusions based on the mean responsiveness presented here should be drawn with caution. Especially, we lack information on responses in net primary production, and it is likely that the absolute responsiveness of net ecosystem

CO_2 fluxes would differ from that of soil CO_2 emissions alone. Finally, none of the nine experiments included interactive effects with other climate drivers, which have previously been shown to be important for the combined response to multiple changes (Larsen et al., 2011).

5 Conclusions

With respect to environmental variables that seemed to influence the magnitude of GHG fluxes across the nine sites, temperature was identified as a main driver of CH_4 emissions across the peatland sites. In contrast, net CH_4 uptake at the temperate shrublands sites correlated negatively with the soil carbon stock, either due to a indirect effect via soil moisture or because soil carbon acted as a source for methanogenesis. Nitrate availability seemed to be a driver of N_2O emissions at both peatland and shrubland sites, as the highest N_2O efflux within each ecosystem type occurred at the site, which had the highest NO_3^- concentrations in soil water. Soil respiratory CO_2 emission showed maximum annual values at the temperate sites with mean annual temperature of 8–10 °C. Outside this range, in each end of our temperature gradient, soil respiration was either limited by low temperatures at the subarctic site SE-Sto or by low summer soil moisture at the Mediterranean site ES-Gar.

In terms of total climate forcing response, prolonged drought and long-term (> 30 yr) drainage consistently reduced the soils' contribution to global warming. However, substantial amounts of CO_2 were presumably lost from the drained peatland immediately after drainage. Furthermore, N addition to peatlands caused a considerable increase in the global warming contribution from these soils, primarily due to the response of CH_4 emissions to $NaNO_3$ addition at one site. Finally, experimental warming had no effect on any of the three GHGs, which could be explained by a modest temperature increase of 0.5 °C imposed by the passive night-time warming treatment. When comparing the fractional importance of each GHG to the total numerical global warming response, then the change in CO_2 efflux dominated the response in all treatments, except for NO_3^- addition where change in CH_4 emissions accounted for the major part. Therefore, within the applied range of water, temperature and nutrient manipulations, soil CO_2 emission plays a dominant role with respect to ecosystems' effect on global warming.

Supplementary material related to this article is available online at: http://www.biogeosciences.net/9/3739/2012/bg-9-3739-2012-supplement.pdf.

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Supplementary material

Table S1. Characteristics of the upper soil layer, generally the O-horizon, at nine experimental sites depending on treatment. Data represents annual mean values and standard error in brackets is an indication of the spatial variability at the sites.

Site	Treatment	Depth cm	Bulk density g cm^{-3}	pH(H_2O) [a]	Total C % by weight	Total N % by weight	Soil water NH_4^+ mg N L^{-1}	Soil water NO_3^- mg N L^{-1}	Soil NH_4^+ [b] µg N g^{-1} dry soil	Soil NO_3^- µg N g^{-1} dry soil	Microbial biomass C mg C g^{-1} dry soil
EE-Män	Control	0-15	0.01	3.4	53	0.6	4.07 (*2.70*)	2.55 (*0.23*)	3.5 (*2.4*)	2.2 (*0.2*)	na
	Drainage		na	2.2	43	1.2	4.25 (*1.10*)	1.28 (*1.11*)	3.4 (*0.9*)	1.0 (*0.9*)	na
SE-Sto	Control	0-10	0.10	4.0	50	1.2	na	na	na	na	na
SE-Fäj	Control	0-10	0.02	3.9	40	1.5	0.63 (*0.55*)	0.52 (*0.19*)	na	na	na
	NH_4NO_3		na	3.9	na	na	na	0.92 (*0.31*)	na	na	na
UK-Whi	Control	0-10	0.08	3.7	46	1.1	0.17 (*0.22*)	0.05 (*0.09*)	26.3 (6.9)	0.16 (*0.02*)	na
	NH_4		0.12	3.7	47	1.3	0.87 (*0.37*)	0.12 (*0.13*)	85.9 (*42.4*)	0.07 (*0.02*)	na
	NO_3		0.11	4.0	49	1.4	0.29 (*0.34*)	0.44 (*0.51*)	63.1 (*7.8*)	0.21 (*0.05*)	na
UK-Clo	Control	0-9	0.09	3.9	45	1.2	0.23 (*0.17*)	0.09 (*0.06*)	5.0 (*2.8*)	0.4 (*0.7*)	2.2 (*0.7*)
	Drought		na	3.9	na	na	0.32 (*0.25*)	0.15 (*0.16*)	3.1 (*1.7*)	0.3 (*0.4*)	4.0 (*1.8*)
	Warming		na	3.9	na	na	0.17 (*0.11*)	0.03 (*0.03*)	5.9 (*3.0*)	0.4 (*0.6*)	2.5 (*0.4*)
DK-Mol	Control	0-3	0.21	3.7	20	1.1	0.87 *(0.44)*	1.55 *(0.69)*	10.1 (8.0)	2.4 (2.7)	0.6 (0.2)
	Drought		0.20	na	21	1.1	1.26 *(0.67)*	1.24 *(0.83)*	5.6 (*2.7*)	2.8 (*2.7*)	0.6 (0.2)
	Warming		0.23	na	19	1.0	1.59 *(0.78)*	1.66 *(1.29)*	8.3 (*4.8*)	4.0 (*4.0*)	0.5 (0.2)
DK-Bra	Control	0-2	0.08	3.1	23	1.2	7.45 (*3.76*)	6.79 (*3.77*)	10.8 (*3.9*)	2.3 (*1.7*)	1.1 (0.2)
	Drought		na	na	na	na	9.91 (*5.95*)	5.52 (*3.95*)	na	na	na
	Warming		na	na	na	na	8.46 (*5.12*)	6.55 (*2.54*)	na	na	na
NL-Old	Control	0-4	0.21	4.2	45	1.9	1.13 (*0.30*)	1.10 (*0.29*)	72.9 (*20.3*)	11.4 (*2.9*)	1.3 (*0.1*)
	Drought		0.21	na	42	1.8	na	na	62.5 (*15.8*)	7.7 (*2.4*)	na
	Warming		0.27	na	45	2.0	na	na	69.6 (*20.9*)	12.8 (*3.2*)	na
ES-Gar	Control	0-12	1.20	8.1	1	0.1	0.11 (*0.06*)	<0.02	0.77 (*0.17*)	0.12 (*0.03*)	0.21 (*0.03*)
	Drought		1.29	na	1	0.1	0.12 (*0.04*)	<0.02	0.71 (*0.22*)	0.09 (*0.02*)	0.20 (*0.04*)
	Warming		1.14	na	1	0.1	0.16 (*0.07*)	<0.02	0.84 (*0.16*)	0.07 (*0.03*)	0.21 (*0.01*)

na, not assessed

[a] Soil pH was measured in 1 M KCl at EE-Män and in 0.01 M $CaCl_2$ at DK-Mol and DK-Bra. These extraction agents result in lower pH values compared to soil suspended in water.

[b] The extraction agent varied with site, thus soil NH_4^+ data should only be used to compare treatments at the site level

Table S2. Characteristics of the lower soil layer, generally below the O-horizon, in untreated control plots at seven experimental sites.

Site	Bulk density $g\ cm^{-3}$	$pH(H_2O)$ [a]	Total C % by weight	Total N % by weight
EE-Män	1.22	3.4	44	0.64
UK-Whi	0.08	3.7	46	0.98
UK-Clo	0.86	4.0	19	0.51
DK-Mol	1.41	4.3	1	0.06
DK-Bra	1.17	3.5	3	0.18
NL-Old	1.14	3.9	2	0.08
ES-Gar	na	8.3	na	na

na, not assessed

[a] Soil pH was measured in 1 M KCl at EE-Män and in 0.01 M $CaCl_2$ at DK-Mol and DK-Bra. These extraction agents result in lower pH values compared to soil suspended in water.

Table S3. Aboveground biomass C and N, total N deposition, nitrate leaching and mean annual water table depth at nine experimental sites depending on treatment.

Site	Treatment	Aboveground biomass C	Aboveground biomass N	Total N deposition [a]	NO_3^- leaching	Water table depth
		g C m^{-2}	g N m^{-2}	g N m^{-2} yr^{-1}	g N m^{-2} yr^{-1}	cm below surface
EE-Män	Control	na	na	0.8	na	15
	Drainage	na	na	na	na	38
SE-Sto	Control	na	na	0.2	na	22
	NH_4NO_3	na	na	4.2	na	na
SE-Fäj	Control	na	na	1.5	na	4
	NH_4NO_3	na	na	5.5	na	na
UK-Whi	Control	2684	49.8	0.8	na	12
	NH_4	2435	59.2	6.4	na	na
	NO_3	2354	54.6	6.4	na	na
UK-Clo	Control	1790	34	1.8	0.4	30
	Drought	na	na	na	0.3	na
DK-Mol	Control	650	9	0.9	1.2	na
	Drought	na	na	0.9	0.8	na
	Warming	na	na	na	0.5	na
DK-Bra	Control	343	11	1.7	0.3	na
	Drought	na	na	na	0.3	na
	Warming	na	na	na	0.2	na
NL-Old	Control	584	10	2.2	1.5	na
	Drought	na	na	na	1.5	na
	Warming	na	na	na	2.7	na
ES-Gar	Control	360	5	0.8	na	na

na, not assessed

[a] Total N deposition was derived from Williams et al. (1999) at EE-Män, Persson et al. (2004) at SE-Sto and SE-Fäj, Bealey et al. (2003) at UK-Clo, Avila et al. (2009) at ES-Gar and bulk deposition collectors at UK-Whi, DK-Mol, DK-Bra, NL-Old and ES-Gar

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Keskkonnamuutustele kohanemise tippkeskus (F11100PKTF)

Taimede stressitaluvuse ökofüsioloogia (SF1090065s07)

NitroEurope IP. The nitrogen cycle and its influence on the European greenhouse gas balance. A1.4.4 (juht) ja A2.3 (teadur) (8-2/T6018PKPK06)

LIST OF PUBLICATIONS

Publications indexed in the ISI Web of Science database:

Portillo-Estrada M, Korhonen JFJ, Pihlatie M, Pumpanen J, Frumau AKF, Morillas L, Tosens T, Niinemets Ü (2013) Inter- and intra-annual variations in canopy fine litterfall and carbon and nitrogen inputs to the forest floor in two European coniferous forests. Annals of Forest Science doi: 10.1007/s13595-013-0273-0 (in press)

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VIIS VIIMAST KAITSMIST

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ERINEVATE HALJASVÄETISKULTUURIDE BIOPRODUKTSIOON JA MÕJU JÄRGNEVATE KULTUURIDE SAAGILE
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22. aprill 2013

TEA TULLUS

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